PHOTOSYNTHESIS AND GROWTH IN *PINUS RADIATA* D. DON AS AFFECTED BY ENVIRONMENTAL FACTORS AND INHERENT QUALITIES

by

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Thesis submitted for the degree of Doctor of Philosophy in the Australian National University.

28 November 1969

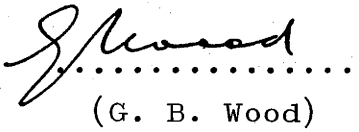
ORIGINALITY OF THESIS

The multiple regressions of the data presented in Studies 6-8 were analysed by computer using a program developed by Mr G.A. McIntyre, Division of Mathematical Statistics, C.S.I.R.O., Canberra.

The microscopic slides of needle sections, photographs of which are presented in Studies 2 and 6, were prepared by a Technical Assistant of the Botany Department, A.N.U., Canberra.

Mr J.P. Wood determined the concentration of carotenoids in needles sampled from seedlings in the 'CERES' Phytotron study (Study 2).

With these exceptions, the work described in this thesis is original and was done without collaboration.



(G. B. Wood)

28 November 1969

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ACKNOWLEDGEMENTS

This project was carried out during tenure of an Australian National University Research Scholarship. I wish to express my sincere appreciation to the University for financing the project and to Professor L.D. Pryor for making freely available the facilities of the Botany Department.

It is a pleasure to acknowledge the encouragement, advice and criticism tendered by my supervisors Drs D.M. Paton, E.G. Brittain and M.J. Aston, to each of whom I am indebted. Special thanks are due to Mr R.R. Willing for valuable advice on plant propagation techniques and for assistance both in the field and in the glasshouse.

The assistance of the following is also gratefully acknowledged: Dr J.H. Fielding and Mr Alan Brown of the Forest Research Institute, Canberra, for helpful discussions and the supply of cross-pollinated seed; the Department of the Interior, A.C.T., for access to the A.C.T. forests and nurseries; staff members of the Physics and Chemistry workshops of the A.N.U. for the construction, maintenance and repair of equipment; Dr L.T. Evans for placing the facilities of the C.S.I.R.O. phytotron 'CERES' at my disposal; staff members and fellow students of the Botany Department, A.N.U., and staff members of the Divisions of Plant Industry and of Land Research and Regional Survey,

C.S.I.R.O., Canberra, for helpful discussions, advice and assistance; the Visual Aids Unit of the A.N.U. for assistance with the preparation of illustrations; and Mrs J. Piper and Mrs B. Driver for invaluable typing assistance.

Finally I wish to express my debt to my wife and children for their self-sacrifice, encouragement and support throughout the period of the project.

ABBREVIATIONS AND SYMBOLS

atm	atmosphere
A.C.T.	Australian Capital Territory
A.E.S.T.	Australian Eastern Standard Time
A.N.U.	Australian National University
[Ca]	concentration of chlorophyll-a
[Cb]	concentration of chlorophyll-b
[Ca+b]	concentration of total chlorophyll
c.c.	cubic centimetre
cm	centimetre
C.S.I.R.O.	Commonwealth Scientific and Industrial Research Organisation
^{o}C	degree centigrade
^{o}K	degree absolute (Kelvin)
dm	decimetre
F.A.A.	formalin-aceto-alcohol preservative
FC	field capacity
ft	feet
ft.c.	foot candle
F.Wt.	fresh weight
g	gram
hr	hour
I.R.G.A.	infra-red gas analyser
l	litre
LB	artificially lit growth cabinet without humidity control
L.S.D.	least significant difference
mb	millibar

M.C.	moisture content
mg	milligram
ml	millilitre
mm	millimetre
mμ	millimicron
mV	millivolt
net P	net photosynthesis
NS	not significant
O.D.Wt.	oven dry weight
oz	ounce
p	probability, or level of significance
p.	page
p.p.m.	parts per million
P/R	ratio of rate of photosynthesis to rate of dark respiration
P/T	ratio of rate of photosynthesis to rate of transpiration
PWP	permanent wilting percentage
r	correlation coefficient
r.p.m.	revolutions per minute
R/S	root/shoot ratio
RWC	relative water content
sp.	species
S.T.P.	standard temperature and pressure
μ	microns
μV	micro volts
>	greater than
≫	very much greater than
⩾	greater than or equal to
<	less than

ABSTRACT

Morphological and physiological responses of *Pinus radiata* to light, temperature, and soil moisture were examined on seedlings grown under glasshouse conditions in both controlled and uncontrolled environments. Each environmental factor profoundly affected seedling development and physiology. Many responses observed under conditions of environmental stress appeared to be compensatory in nature. This suggests possible reasons why the species has the capacity to exist under a wide range of diverse environments. Research is necessary to determine whether compensatory responses are unusual in the genus before one might suggest that they provide an explanation, at least in part, for the outstanding performance of *P. radiata* as a plantation exotic.

Studies were also made of the morphology and physiology of foliage in the crown of a *P. radiata* sapling in a forest plantation closing canopy. The most active photosynthetic region of the tree was associated with 1- and 2-year-old needles in the upper levels of the crown where light was less often limiting for photosynthesis, pigment concentration was high, and leaf water deficits at dawn were lower than elsewhere on the tree. If these relationships apply generally, the forester will have a physiological basis on which to examine critically the empirical schedules currently

in use for pruning and thinning forest stands of the species.

The results contribute to knowledge of the response of P. radiata to the physical environment. The necessity for follow-up research is stressed and some fertile areas for future research are suggested.

I INTRODUCTION

ECONOMIC IMPORTANCE OF PINUS RADIATA

Economic considerations prompted the choice of Pinus radiata D. Don as the study species. It is an unimportant species in its natural habitat, being restricted to an area of approximately 20,000 acres on the Californian coast (Beekhuis and Will, 1965; Forde, 1966), but it thrives as an exotic mainly in the temperate zone of the Southern Hemisphere. The total plantation area of the species in the world is approximately two million acres, distributed between countries as follows - New Zealand 37 per cent, Chile 32 per cent, Australia 20 per cent, Spain 8 per cent, South Africa 3 per cent (Raupach, 1967). The species was introduced into Australia over a century ago, and today, plantations extend from $27\frac{1}{2}^{o}$S latitude to 43^{o}S latitude. P. radiata dominates the commercial exotic pine market in both Australia and New Zealand. This is due largely to its exceptionally high rate of growth, its great flexibility in management and an apparent ability to tolerate a range of diverse environments.

IMPORTANCE OF TREE PHYSIOLOGICAL RESEARCH TO SILVICULTURAL PRACTICE AND FOREST PRODUCTIVITY

The economic management of a forest demands that the stands be manipulated through pruning, thinning and other cultural techniques so that the rate of growth

and quality of the forest are governed. This silvicultural manipulation involves a disturbance of microclimatic relationships which modifies the physiological responses of the trees. Simultaneously, the natural development of the forest generates its own changes in the ecosystem which, in turn, affect the further development of the forest. The whole is a complex and dynamic entity, but the forester seldom appreciates it as such. His interest is largely confined to the quantity and quality of production and insufficient attention is given to the physical and biological conditions which determine production. The result is that the majority of his management decisions are based on empirical knowledge. The limitations of this type of knowledge in improving cultural methods are well known in crop agronomy (Gast, 1965).

Plant and tree growth is dependent primarily on the efficiency of the photosynthetic process in converting atmospheric CO_2 and water in the presence of light into carbohydrates, and subsequently into usable forest produce. The stagnation, poor growth, or failure of any forest is due most often to a physiological disturbance of the trees, which is reflected in a gross inefficiency of the photosynthetic system.

In view of the economic importance of P. radiata, it is surprising that information currently available on the physiological responses of the species to the environment is fragmentary. This is apparent on reference to recent bibliographies (Scott, 1960; Pert, 1963; Marris, 1965, 1966). It implies that the forester

lacks a sound understanding of the relationship between the species and its environment. Thus, it may be impossible for him as a plantation manager to realise the aim of creating the most favourable conditions for tree growth through breeding and cultural treatment.

SCOPE AND OBJECTIVES OF THE INVESTIGATION

Choice of subject was determined by the fact that photosynthesis is a most important physiological process in a green plant. It provides the source of chemical energy and the substrates for all subsequent biosynthesis by the plant. Dry matter production is directly dependent on the total amount of net photosynthesis which varies with light, temperature, soil moisture and with changes in other factors of the environment. The three variables specifically mentioned above were selected for detailed study as they largely determine the performance of a tree species on a new site and/or the growth and development of an established crop. Plant nutrition was not covered as research in this field is well established. The possible effect of photoperiod on the photosynthetic performance of P. radiata was excluded from the project as its influence on the height growth and dry matter production of the species is weak (Downs and Piringer, 1958; Vaartaja, 1959; Downs, 1962).

The plant material consisted of seedlings and 6- to 8-year-old plantation trees as practical experience has shown that the most crucial period in the life of a forest plantation is that from establishment to the time immediately following first thinning (approximately 10

years in Australia). During this period, site conditions and management and silvicultural practices largely determine the ultimate potential of the forest.

The specific objectives of the research were:

Laboratory: (i) to investigate photosynthesis and growth in P. radiata seedlings under a range of light, temperature and soil moisture conditions, and, simultaneously, to attempt to find reasons why the species has an outstanding capacity to exist under a wide range of diverse environments;

(ii) to develop a technique for examining the photosynthetic activity of foliage of a forest tree using detached shoot material.

Field: (iii) to assess for a single forest tree sampled at one point in time, the spatial distribution of foliage in the crown and spatial variation in foliage morphology, pigment content, relative water content, and photosynthetic activity.

II LABORATORY STUDIES

MATERIALS AND METHODS - GENERAL

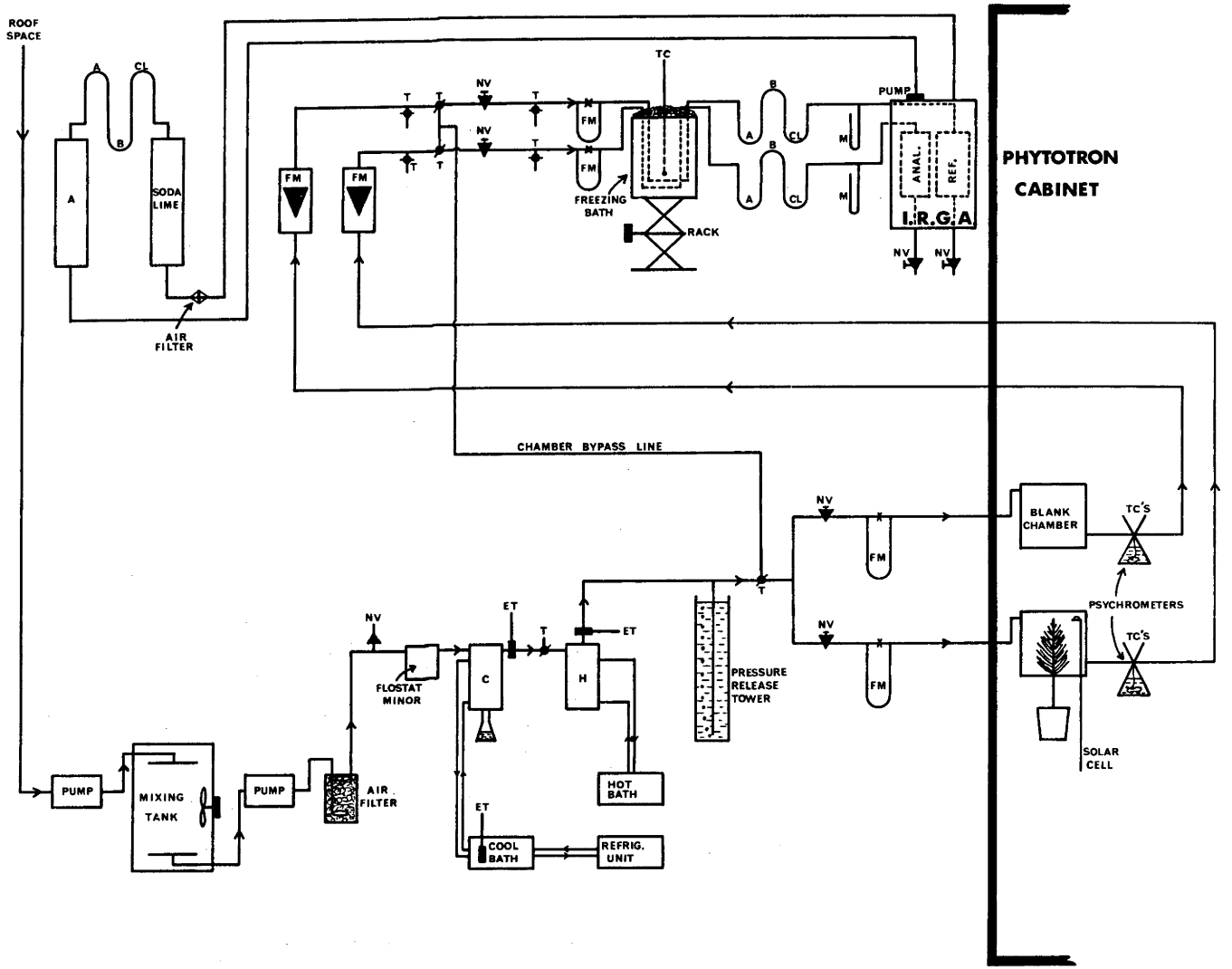
Plant material: The majority of the P. radiata seedling material used in the laboratory studies was raised from a single batch of seed resulting from the cross-pollination of two selected parents. The seed in all cases was given six weeks moist cold storage at $4^{o}C$ before being sown broadcast in perlite or in a 1:1 perlite/vermiculite mixture. When the seedlings were approximately 3" high, they were transplanted into polystyrene pots containing either soil (glasshouse studies) or the perlite/vermiculite mixture (phytotron study). The soil used was a mixture of loam, river sand, and peat in the ratio 12:3:3 plus 4 oz John Innes base fertiliser (NPK based on organic nitrate) per bushel of mix.

Instrumentation: (a) CO_2-exchange - An Infra Red Gas Analyser (Type SB/K), manufactured by the Infra-red Development Company, England, and set up for differential measurement, was used. The instrument measured a differential of $\pm$ 15 p.p.m. CO_2 from a centre zero with a sensitivity of 1 p.p.m. It was found by test to have a linear response, and was calibrated periodically by purging one absorption tube with CO_2-free air and the other with air of known CO_2-concentration. The analyser was used in an open system (Figure 1), the background CO_2-concentration being atmospheric. Interference due to water vapour absorption was eliminated by drying the gases completely prior to their entering the analyser.

FIGURE 1 Schematic representation of the gas circuit used to measure CO_2- and H_2O-vapour exchange in the study material

Legend:

A - $CaCl_2$)
B - $CaSO_4$) Drying agents
CL - $Mg(ClO_4)_2$)
C - Condensing unit
ET - Electric thermometer
FM - Flow meter
H - Heating unit
M - Manometer
NV - Needle valve
T - Tap
TC - Thermocouple


ROOF SPACE
A
CL
B
SODA LIME
AIR FILTER
FM
T
NV
TC
FREEZING BATH
RACK
PUMP
ANAL.
REF.
I.R.G.A
PHYTOTRON CABINET
CHAMBER BYPASS LINE
BLANK CHAMBER
TC'S
PSYCHROMETERS
SOLAR CELL
PRESSURE RELEASE TOWER
FLOSTAT MINOR
ET
C
H
HOT BATH
COOL BATH
REFRIG. UNIT
PUMP
MIXING TANK

The output was displayed on a 'Honeywell' multi-channel potentiometric recorder.

(b) Water vapour exchange - The rate of water loss by the plant was monitored simultaneously with CO_2-exchange by using two psychrometers, one each on the exhaust lines of the blank and assimilation chambers (Figure 1). The dry bulb thermocouple of the plant chamber psychrometer also served the function of monitoring the chamber bulk air temperature, a continuous record of which was displayed on the 'Honeywell' recorder.

(c) Environmental control - Control of light intensity and temperature in the assimilation chamber was achieved by installing the chamber inside a 'Phytotron' LB cabinet of the type described by Morse and Evans (1962) and Pescod et al. (1963). The cabinet provided constant temperature control ($\pm 1^{\circ}C$) at any temperature in the range $0-35^{\circ}C$. It was artificially lit by an arched sealed canopy of 28 TL-33 high output, internal reflector, fluorescent lamps, and four incandescent lamps, which were connected to a time switch for photoperiod control. Light intensity was regulated by switching out pairs of the fluorescent tubes, and was monitored with a Hoffman silicon solar cell (No. 110C). The cell was covered with a diffusing dome to give cosine correction (see Fleming and Trickett, 1966), and was calibrated on the 'Honeywell' recorder against a Moll-Gorczynski solarimeter (Kipp and Zonen) and an Eel 'Lightmaster' photometer for the spectral band 400-700 mμ. The cell was insensitive to temperature over the range $0-35^{\circ}C$. The incandescent lamps were switched off during the measurement of photosynthesis

as there was no simple practical means of controlling their intensity. This avoided light quality changes when photosynthesis was being measured over a range of light intensities.

(d) Assimilation chamber - The assimilation chamber was made from 3/16" clear perspex rolled into a cylinder 10" in diameter and 18" high. The ends were sealed with discs of 3/8" perspex, the top disc being fixed. The basal disc was two-segmented with a 1 1/4" diameter hole drilled in the centre for enclosing a tree branch or stem of a seedling. It was sealed to the chamber with wing nuts, 'O' rings, and plasticine. Air was introduced into the top of the chamber through an 8" diameter circular distributor of copper tubing, drilled in a spiral arrangement with 1/8" diameter holes at horizontal intervals of 1". The air was exhausted from the base of the chamber via an identical perforated receptor.

(e) Gas circuit - The gas circuit is illustrated in Figure 1. Except for minor modifications, it is the same as that described by Cameron (1964). All connections were of glass or copper tubing. The circuit was an open system drawing air from the roof space of the Botany Building. After stirring the air, filtering it, and smoothing its flow rate of pump oscillations, it was cooled to approximately 7°C to condense some of its contained water vapour, and then rewarmed to the operating temperature (these last two steps in effect reduced and controlled the relative humidity of the air stream). The air stream was then divided into two and metered at equal rates into the

assimilation and blank chambers (the purpose of the blank chamber being to equalise the volumes and geometry of the 'analysis' and 'blank' gas lines). The gas streams emerging from the chambers were checked for flow rate (to detect possible chamber leaks) before bleeding off the majority to waste, leaving a small part to be analysed. This part was metered to equalise the flow rate in both lines, and dried by passing it through condenser tubes packed in a dry ice/absolute alcohol mixture at a temperature $<-15^{o}C$ and then over chemical absorbents. Finally, it was passed into the analysis and reference tubes of the CO_2-analyser, which were maintained under equal pressure, and exhausted to the atmosphere. A chamber bypass line enabled the instrument zero to be recorded when desired.

The liquid flow meter manometers in the circuit were calibrated by water displacement under a constant pressure head. In a given experiment, the flow rate through the assimilation chamber was held constant as photosynthesis varies with the rate of air supply.

(f) Analogue data recording system - Voltages generated by the various sensing elements in the circuit (solar cell, CO_2-analyser, thermocouples) were recorded continuously on a 'Honeywell' multi-channel 3mV potentiometric recorder and read by eye. The reproducibility of the recorder was checked by applying a constant voltage across one set of terminals and recording continuously for a period of 30 minutes. It was established that the trace was reproducible within

0.1 chart units, i.e., the recorder could distinguish between voltages differing by as little as 3μV.

Expressing the rate of gas exchange: Various indirect methods can be used to estimate the amount of foliage taking part in gas exchange in Pinus species, e.g., surface area, volume, fresh and dry weight, total length or number of needles, and chlorophyll content (Verduin, 1953, 1959). Surface area is the index most commonly used in studies of broadleaf plant species, but, quite apart from problems of measurement, it is an unsatisfactory index for Pinus as the relationship between the total area of leaf surface and the area of the illuminated surface varies in different types of leaf (Sweet and Wareing, 1968). P. radiata has primary leaves, and secondary fascicles containing from 2-5 needles each: the shape of all these leaves is different. The most consistent index of foliage quantity on which to base rates of gas exchange in Pinus is volume (Clark, 1961; Rook, 1965), and this index was used in the majority of studies described.

Computation of gas exchange rates: The data were processed on an IBM 360 computer using a program specially written for the purpose. This program was based on the following equations:

(1) Calculation of the rate of CO_2-exchange

$$\text{Rate of } CO_2\text{-exchange} = \left[\frac{dxSxF}{10^6 xV}\right] \times \left[\frac{px273.16x1976.96}{760 \times T}\right]$$

(mg CO_2/c.c./hr)

where d is the CO_2-differential corrected for the instrument zero in chart units

S is the I.R.G.A. sensitivity/chart unit (p.p.m.)

F is the flow rate in litres/hour

V is the volume of foliage in c.c.

p is the line pressure in mm Hg

T is the absolute temperature (^oK)

and 1976.96 is the density of CO_2 at S.T.P. in mg/l derived from $\frac{\text{Mol.Wt.}}{\text{G.Mol.Vol.}} = \frac{44.011 \times 10^3}{22.262*}$

= 1976.96.

* CO_2 is not a perfect gas - Schaum et al., 1958.

(2) Calculation of the saturated vapour pressure at any temperature (List, 1963, p.350)

$$\text{Log}_{10} e_w = -7.90298\left(\frac{Ts}{T} - 1\right) + 5.02808\ \text{Log}_{10}\left(\frac{Ts}{T}\right) - 1.3816 \times 10^{-7}\left(10^{11.344\left(1 - \frac{T}{Ts}\right)} - 1\right) + 8.1328 \times 10^{-3}\left(10^{-3.49149\left(\frac{Ts}{T} - 1\right)} - 1\right) + \text{Log}_{10} e_{ws}$$

where e_w is the saturated vapour pressure in mb

Ts is the steam point temperature = 373.16^oK

T is the absolute temperature (^oK)

e_{ws} is the saturation vapour pressure of water at temperature Ts = 1013.246 mb

(3) Conversion of the saturated vapour pressure in mb (e_w) to mm Hg (List, 1963, p.59)

$$e' = 0.7500616 \text{ Exp } [2.302585 (\text{Log}_{10} e_w)] \quad \text{(mm Hg)}$$

(4) Calculation of the vapour pressure (e) of air in mm Hg (List, 1963, pp.365-6)

$$e = e' - 0.000660\ p\ (t-t')\ (1 + 0.00115\ t') \quad \text{(mm Hg)}$$

where p is the line pressure in mm Hg
t is the dry bulb temperature of the air in ^{o}C
t' is the wet bulb temperature in ^{o}C
and e' is the saturated vapour pressure in mm Hg at temperature t'

(5) Calculation of the absolute humidity of the air (R.G. Wylie - pers. comm.)

$$X = \frac{0.622e \times 273.16 \times 1.2929}{760 \times (t + 273.16)} \quad \text{(mg/l)}$$

(6) Calculation of the rate of transpiration (T)

$$T = \frac{F(X_1 - X_2)}{V} \quad \text{(mg } H_2O\text{/c.c./hr)}$$

where F is the flow rate in litres/hr

X_1 is the absolute humidity of the air in the analysis line (mg/l)

X_2 is the absolute humidity of the air in the reference line (mg/l)

and V is the foliage volume in c.c.

Note: The line pressure 'p' in equations (1) and (4) was the barometric pressure corrected for the positive pressure in the system which had previously been determined for a range of flow rates.

Notes on the assessment of plant parameters:

Needle length - measured as the length in centimetres from the tip of the needle to the base of the fascicle sheath.

Needle thickness - measured as the length from the middle of the abaxial face to the common axis of the plane faces. Measurements were made with a 'Leitz' 6X optical scale at needle mid-length.

Needle shape - characterised by the ratio of needle length to needle thickness, both parameters being expressed in the same unit.

Needle surface area - calculated by multiplying the length of the needle by 4.1 times its thickness (see Appendix 1).

Fascicle sheath length - where the perimeter of the sheath was frayed and/or irregular, the shortest and longest lengths were averaged.

Foliar angle - measured with a protractor modified for the purpose. The angle measured was that between the stem and leaf axes.

Stomatal rows - occurrence assessed both by counting the total number of rows on the abaxial face of each needle at mid-length (Appendix 2) and by determining the number per mm of needle thickness (it follows from Appendix 1 that the length of the curved abaxial face of a needle is a standard function of needle thickness).

Needle moisture content - foliage weighed fresh and then dried in a forced draught oven at 95°C for 48 hours.

$$M.C.(\%) = \frac{100\ (\text{fresh weight} - \text{oven dry weight})}{\text{oven dry weight}}$$

Foliage relative water content (RWC) - assessed by the procedure outlined in Appendix 4. Briefly, it involved weighing the needles fresh, turgid (after 24 hours immersion of the needle bases in water in the dark), and oven dry, and then applying the formula:

$$RWC = 100\ \left(\frac{\text{fresh weight} - \text{oven dry weight}}{\text{turgid weight} - \text{oven dry weight}}\right)$$

Foliage volume - measured by fluid displacement using a slightly modified version of the volumeter and technique of Clark (1961). Foliage volume was calculated as the difference between the volume of the shoot plus and minus its foliage (needles severed at the top of the fascicle sheath). Accuracy of the volumeter was $\pm$ 1 per cent of true volume. Absolute alcohol rather than water was used as the displacement fluid to avoid the formation

of air bubbles on the needles and to hasten drying after the initial immersion of the complete shoot. A preliminary trial revealed that absolute alcohol, a dehydrating agent, had no effect on the volume of a pine shoot during the short period involved in measurement.

Seedling height - the height from the cotyledonary scar to the base of the terminal rosette, or to the tip of the apical bud if a resting bud had formed.

Stem basal diameter - measured with a vernier calipers at a point 1 cm below the cotyledonary scar. Two readings were taken at right angles to each other and averaged - this procedure minimises the error due to stem eccentricity should it occur.

Height/diameter ratio - this ratio is commonly used to characterise the general form of an erect plant.

Distribution of stem fascicles - assessed by counting the number of fascicles on a specified section of stem and expressing the result as the number per unit length of section.

Branching - the extent of branching of a seedling was assessed both by counting its total number of long shoots and by expressing the oven dry weight of these shoots as a ratio of the total oven dry weight of the stem.

Stockiness rating - the ratio of the oven dry weight of the shoot to shoot height (cf. Shirley, 1945;

Steinbrenner and Rediske, 1964). The ratio is commonly used to assess quantitatively the stockiness of erect plants.

Plant pigments - the methods used in needle preparation, pigment extraction, and spectrophotometric analysis are described in Appendix 3. Briefly, the pigments were extracted in 80 per cent acetone (acetone/distilled water - v/v) and the optical densities of the filtered extracts were read at 450, 645, and 663 mμ. The concentrations of chlorophyll and the carotenoids were determined using the formulae of Arnon (1949) and Jaspars (1965) respectively.

Dry matter production and root/shoot ratio - stems were severed from roots in the region of the hypocotyl and the roots were washed free of the rooting medium. The two lots of plant material were then oven dried and weighed.

Note: Preliminary trials revealed that the various ratios referred to in the notes above were unaffected by ontogenetic development in seedlings 5 to 8 months old (Table 1). As the effects of environmental factors on the ratios were examined in 7- to 8-month-old material, it may be assumed that any trends observed are due to the treatments imposed.

Soil moisture content of drained pots: The soil moisture content was monitored using porous conductivity blocks constructed of monel metal mesh electrodes insulated with fibre glass and impregnated with gypsum ('Moistest' blocks sold by H.P. Mulligan Pty. Ltd. of Sydney). Resistance measurements were made with a Soil

TABLE 1 Variation due to ontogenetic development in the height/diameter (H/D) and root/shoot (R/S) ratios, and in the ratio of the oven dry weight of long shoots (branches) to the oven dry weight of the whole stem (LS/S) of P. radiata seedlings from the 3rd to the 8th month after germination. Growing conditions: day temperature - 24°C; night temperature - 19°C; light - full sunlight; photoperiod - 16 hours.

Ratio	Seedling age (months)						L.S.D. 5%
	3	4	5	6	7	8	
			(± 3.3)				
H/D	40.8	55.2	65.5	62.1	66.1	62.7	10.4
			(± 0.04)				
R/S	0.43	0.21	0.21	0.25	0.22	0.27	0.13
			(± 0.03)				
LS/S	0.06	0.12	0.18	0.20	0.22	0.22	0.10

Moisture and Temperature Bridge, Model 200A, built by National Instruments Pty. Ltd. of Sydney. Each block was calibrated individually against soil moisture content using the technique described by the U.S. Department of Agriculture (1962). Calibration was undertaken on the drying curve since conductivity blocks display marked hysteresis effects (Cope and Trickett, 1965). The blocks were both calibrated and used at 25°C. Correction of block resistance for temperature was thus unnecessary (see Rahman, 1967).

Leaf anatomical studies: Needle specimens were preserved in F.A.A. until required for microscopic examination. The procedures used in dehydration, infiltration, embedding, sectioning, and staining were based on standard methods outlined in Johansen (1940) and Purvis et al. (1964). Specimens, embedded in wax, were sectioned on a sliding microtome, and stained with safranin and light green. The prepared slides were examined under a Reichert 'Zetopan' microscope to which was attached photomicrographic equipment.

STUDY 1

PHOTOSYNTHETIC RESPONSE OF SEEDLINGS TO LIGHT AND TEMPERATURE

The specific effects of light and temperature on the photosynthetic process in plants are complex, but an examination at different temperatures of the characteristics of the light response curves of photosynthesis yields useful information on some of these effects (Björkman and Holmgren, 1963). Thus, the aim of the study was to establish for P. radiata seedlings a set of light response curves covering a range of temperatures.

MATERIALS AND METHODS

Twenty plants of uniform development were selected from a population of eight-month-old seedlings raised from cross-pollinated seed under glasshouse conditions as described earlier. The stock was well watered at all times. Seedlings were allotted at random to one of five temperature treatments, viz., 11°, 17°, 23°, 29°, or 35°C, giving four replicates per treatment. Prior to gas exchange measurement, each seedling was pre-conditioned for one hour at its allocated temperature. A preliminary trial had indicated a slight diurnal variation in seedling CO_2-exchange capacity (Figure 2); hence, gas analysis measurements were commenced on Replicates 1 - 4 at 1000, 1200, 1400, and 1600 hours respectively to minimise any effects

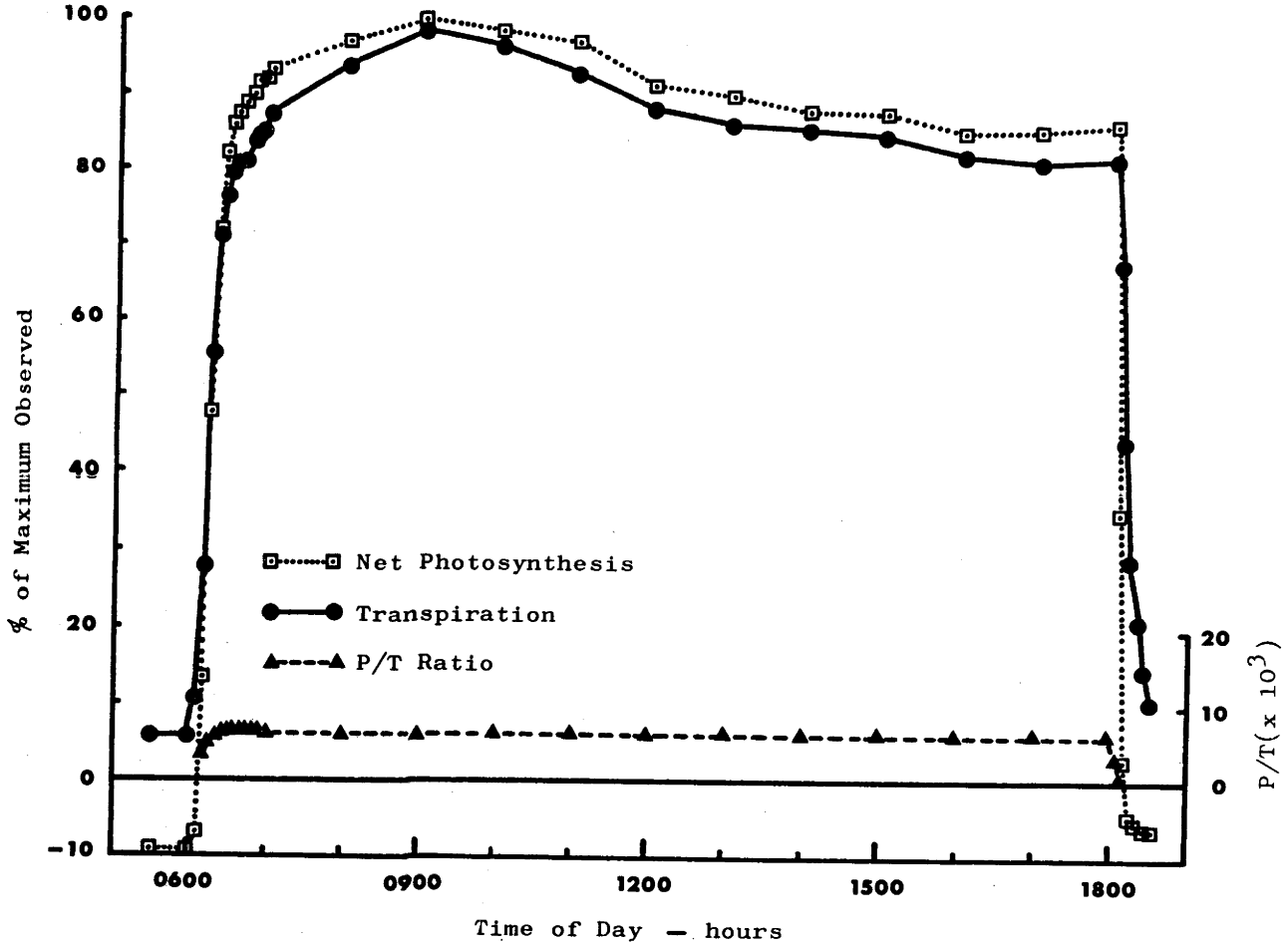


FIGURE 2 : Diurnal variation in the rate of net photosynthesis and transpiration and in the P/T ratio of a P. radiata seedling growing in a controlled environment, viz., 25°/16°C, 2500 ± 100 ft.c., 12 hour photoperiod. Each point is a mean of five replicates.

that this variation (which may have been due to variation in ambient CO_2-concentration and plant water status) may have had on the results.

The procedure with each seedling was to enclose the upper third of the shoot in the assimilation chamber, and allow its rate of net photosynthesis to reach a steady state under full light (3000 ft.c.). After equilibration, the light intensity was reduced to the next level and the new rate of photosynthesis recorded - and so on, descending through the light intensity series. Dark respiration was also measured. The above method of defining the light response curve by descending from a high to low light intensity is conventional practice (Wassink et al., 1956; Ludwig et al., 1965) as equilibration times are shorter on a descending than on an ascending series. The method ignores the possible effect of the previous light intensity on CO_2-uptake at the new intensity. A constant air flow rate was used throughout. Because ambient temperature was varied, the water vapour pressure varied from 8 mm Hg at 11°C to 17 mm Hg at 35°C. The vapour pressure gradient from the ambient air to the mesophyll tissue could not be estimated as the method used to record leaf temperature (fine wire thermocouples inserted in the leaf) was unreliable.

Light response curves were constructed from the average data of net photosynthesis for each temperature (Table 2, Figure 3). Light compensation points were determined graphically and the initial slopes between 0 ft.c. and 500 ft.c. were calculated assuming that the sections of the curves in this region of light intensity

were linear. The P/R ratio was calculated by dividing the absolute rate of gross photosynthesis at 3000 ft.c. (assuming gross photosynthesis = net photosynthesis + dark respiration) by the absolute rate of dark respiration.

All the data were subjected to an analysis of variance.

RESULTS

Light compensation point - The compensation point for light increased steadily with rise in temperature (Table 3). Differences between treatments are significant at the 1 per cent level except for the 11° and 17°C treatments which do not differ significantly.

Initial slope - The effect of temperature on the initial slope of the light response curves is significant at the 1 per cent level due mainly to the depressed slope at 35°C (Table 3). The slopes at 11°, 17°, 23°, and 29°C do not differ significantly.

Saturating light intensity - Saturation was achieved in the region 2500-3000 ft.c. at both 11° and 17°C (Figure 3). Quantitative data for saturation at higher temperatures are unavailable as the maximum light intensity possible in the experimental set-up was insufficient to saturate the photosynthetic mechanism at these temperatures.

Rates of net photosynthesis at the various light levels - Temperature had a significant effect ($p < .001$) on the rates of CO_2-exchange over the range of light intensities. The main trends (Figure 4) are:

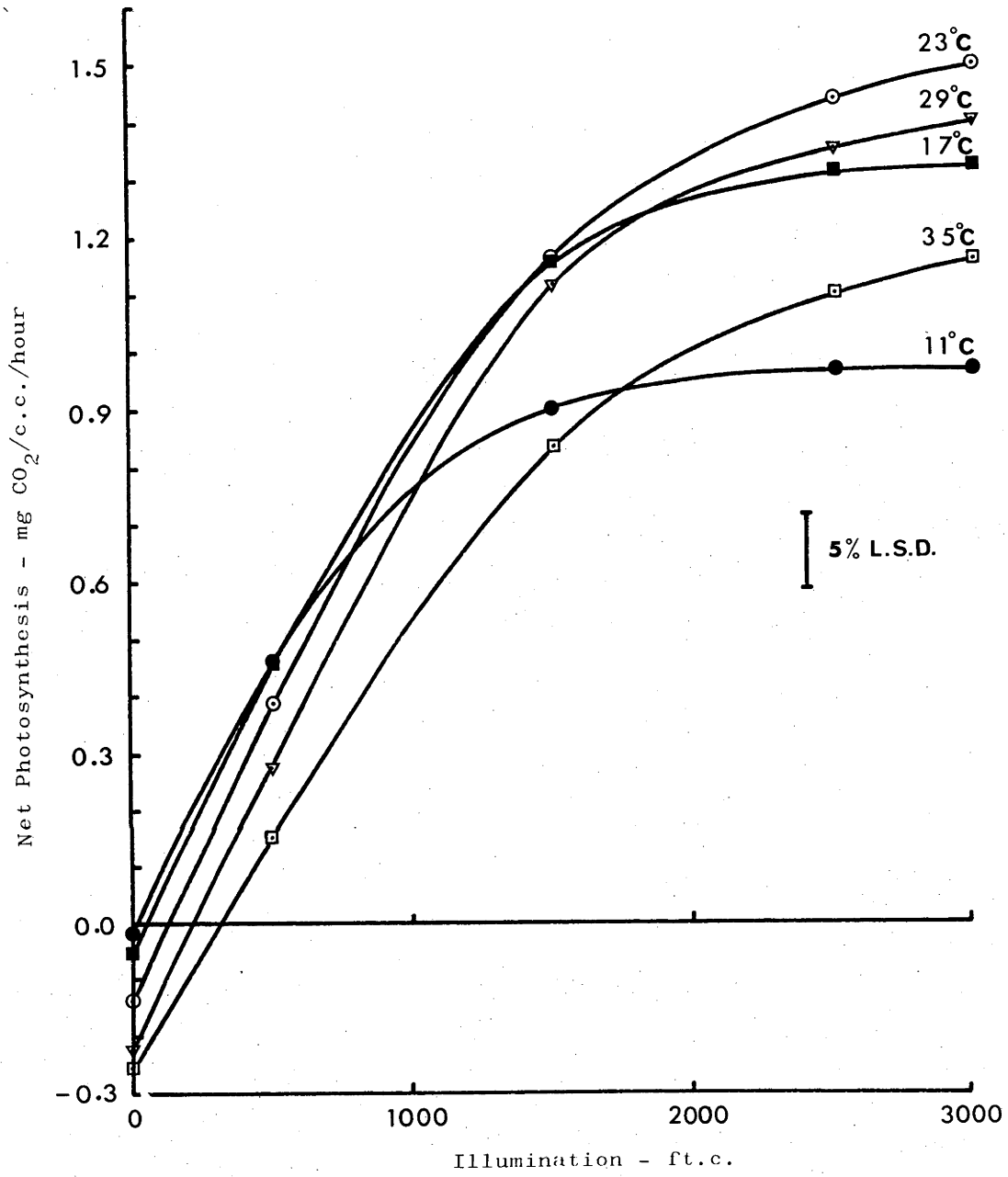


FIGURE 3: Light response curves of net photosynthesis of eight-month-old *P. radiata* seedlings at each of five temperatures. Each point is a mean of four observations.

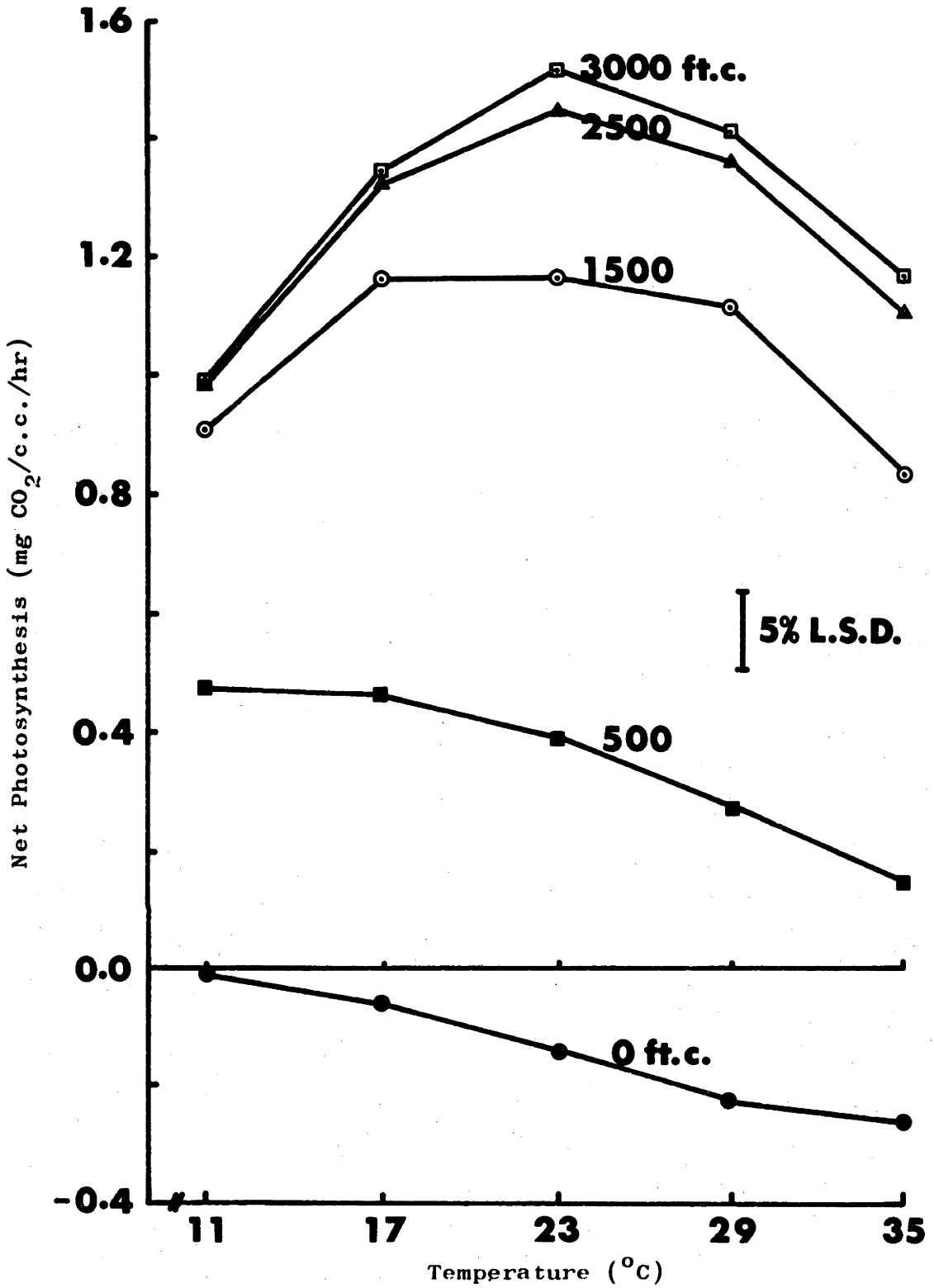


FIGURE 4: Effect of temperature on net photosynthesis of eight-month-old *P. radiata* seedlings at each of five levels of illumination. Each point is a mean of four observations.

TABLE 2 Net photosynthesis (mg CO_2/c.c./hr) of 8-month-old P. radiata seedlings at five light intensities under each of five temperature regimes. Each entry is an average of four observations.

Temperature (°C)	Light intensity (ft.c.)					Mean (± 0.02)
	0	500	1500 (± 0.05)	2500	3000	
11	-0.01	0.47	0.91	0.98	0.99	0.67
17	-0.06	0.46	1.17	1.33	1.34	0.85
23	-0.14	0.39	1.17	1.45	1.52	0.88
29	-0.23	0.28	1.13	1.37	1.42	0.79
35	-0.26	0.15	0.84	1.12	1.18	0.61
Mean (± 0.02)	-0.14	0.35	1.04	1.25	1.29	0.76
5% L.S.D. 0.13			1% L.S.D. 0.17			.1% L.S.D. 0.22

TABLE 3 Light compensation points and initial slopes of the light response curves for 8-month-old P. radiata seedlings at each of five temperatures. Each entry is an average of four observations.

Temperature (°C)	Compensation point (ft.c.) (± 18)	Initial slope* (± 0.07)
11	10	1.94
17	45	2.09
23	125	2.11
29	221	2.01
35	310	1.62
5% L.S.D.	55	0.20
1% L.S.D.	77	0.28
.1% L.S.D.	109	0.40

* Expressed in arbitrary units

(i) dark respiration rate increased significantly with rise in temperature ($p < .001$);

(ii) the rate of net photosynthesis at 500 ft.c. decreased with increase in temperature;

(iii) the rate of net photosynthesis at 1500+ ft.c. was significantly depressed ($p < .001$) at 11^{o} and 35^{o}C relative to that at intermediate temperatures;

(iv) the optimum temperature for net photosynthesis varied with light intensity, being 11^{o}-17^{o}C at 500 ft.c. and rising to 23^{o}C at 3000 ft.c. However, the rate of net photosynthesis at light intensities > 1500 ft.c. was largely unaffected by temperature in the range 17^{o}-29^{o}C.

P/R ratio - The P/R ratio was inversely related to temperature (Figure 5).

DISCUSSION

A factor ignored in later discussion is the effect on results of lack of control of the humidity of the air stream. Vapour pressure differences in the air supply at the various experimental temperatures could conceivably have led to differences in the vapour pressure gradients from the air stream to the intercellular spaces. If this effect occurred, and was substantial, the rate of CO_2-exchange could have been altered by a change in one or more of the diffusive resistances, e.g., stomatal closure due to water stress. This limitation on subsequent discussion should be borne in mind.

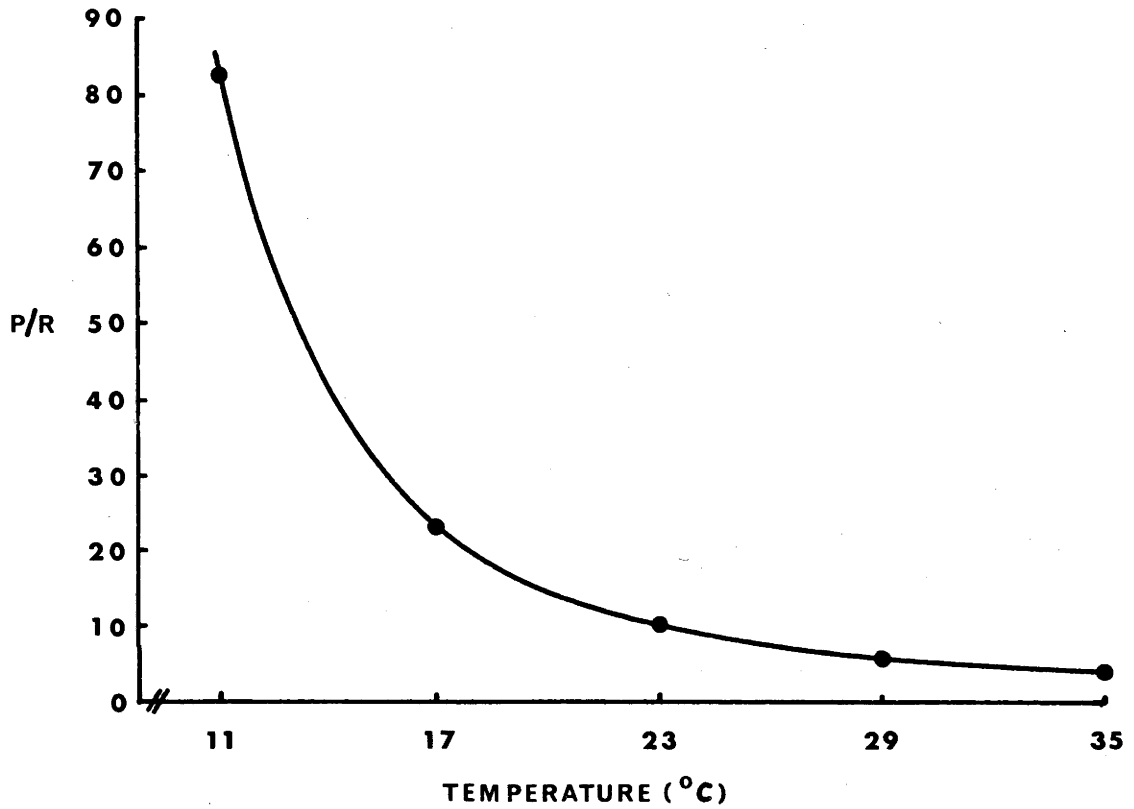


FIGURE 5: Effect of temperature on the P/R ratio of eight-month-old *P.radiata* seedlings, where P is the rate of total photosynthesis at 3000 ft.c. and R is the dark respiration rate.

The response curves illustrated (Figure 3) are of the Bose type (Rabinowitch, 1951), the transition from the linear to the horizontal sections being gradual due probably to both the optical density of the leaf tissue (Talling, 1961) and the density of the foliage layers.

Light compensation point

Assuming an adequate CO_2-concentration, the compensation point for light depends mainly on the rate of respiration and the optical density of the plant specimen (Rabinowitch, 1951). The rise in the compensation point with increase in temperature (Table 3) is a well established phenomenon. It is attributed in this case to an increased rate of respiration as the plant material used was uniform in size, shape, and colour: little variation in the optical density of foliage would be expected. CO_2-concentration was adequate as the compensation point at all temperatures fell in the linear range of the light response curve. This indicates that the diffusion process was not limiting.

Initial slope of curves

The absence of a significant difference in the initial slope of the response curves at 11^{o}, 17^{o}, 23^{o}, and $29^{o}C$ accords with the hypothesis that, within the biokinetic range, the primary photochemical process is independent of temperature (Rabinowitch, 1956; Gaastra, 1962). The significant reduction in slope at $35^{o}C$ indicates a depression in the capacity of the photochemical process of photosynthesis and a temperature dependence of the maximum quantum yield of

photosynthesis. This situation cannot occur within the biokinetic range (Rabinowitch, op. cit.).

Effect of temperature on the rate of CO_2-exchange at different light intensities

The increase in the dark respiration rate with rise in temperature (Figure 4) is a response well known in a wide variety of plants. A point of some consequence is the dependence of the dark respiration rate of leaves on the preceding light environment (Ludwig et al., 1965) presumably as a result of different photosynthetic rates and consequent accumulations of photosynthate. This may have some bearing on the rates of dark respiration observed, e.g., the differences between the treatments at higher temperatures are probably underestimated and those at lower temperatures overestimated.

The fall in the rate of net photosynthesis at 500 ft.c. with rise in temperature (Figure 4) occurs because temperature has a marked effect on the respiration rate but a negligible effect on the gross photosynthetic rate at this low light intensity (at which the photochemical reaction is limiting - Figure 3). The change in the optimum temperature for net photosynthesis over the range of light intensities is explained by the fact that light intensity predominantly influences the rate of photosynthesis, whereas temperature influences both photosynthesis and respiration in different ways.

The wide range observed in the optimum temperature for net photosynthesis at the higher light intensities

(Figure 4) is probably due to a limitation of photosynthesis by the CO_2-diffusion process, which is only slightly influenced by temperature. Similar wide ranges have been reported by Brix (1964) and Whiteman and Koller (1964). The fall in the rate of photosynthesis at either end of the temperature range may be partly due to increases in the leaf diffusive resistances. At higher temperatures, increases in both stomatal and mesophyll resistances can occur (Whiteman and Koller, op. cit.; Waggoner and Zelitch, 1965), while at lower temperatures an increase in stomatal resistance is possible (Zelitch, 1965). A depressed activity of enzyme systems at high temperatures and restriction of the diffusion process at low temperatures might also be involved.

The inverse relationship between the P/R ratio and temperature (Figure 5) is often observed because the Q_{10} for photosynthesis is approximately 1 whereas that for respiration is 2-3 (Marshall, 1967). The trend of the P/R curve suggests that, at high temperatures, there may be a marked decrease in food material available for growth and storage.

The finding of most consequence is the limitation of the photochemical partial process of photosynthesis at $35^{o}C$, which suggests that this temperature was supra-optimal for photosynthesis in the study material.

STUDY 2

EFFECT OF LIGHT INTENSITY AND TEMPERATURE ON SEEDLING GROWTH AND HABIT, LEAF MORPHOLOGY, AND GAS EXCHANGE CAPACITY

In the previous study, short term effects of light and temperature on the photosynthetic mechanism in P. radiata seedlings were examined. The aim of this study was to investigate the long term effects of these same factors on some aspects of seedling morphology, physiology, and dry matter production.

MATERIALS AND METHODS

Environmental control facilities

The experiment was conducted under glasshouse conditions in the 'CERES' phytotron, C.S.I.R.O., Canberra, which provided control over temperature and photoperiod. Ambient temperatures were maintained at the day temperature for 8 hours of the light period, and at the night temperature for the remaining 16 hours of each 24-hour cycle. The commonly used 'CERES' thermoperiod of a 5°C depression in night relative to day temperature was used. The temperature of the rooting medium was found to approximate the ambient air temperature, differing both day and night by less than 1°C. The lighting was daylight, being subject to the normal diurnal and seasonal variation. The daylight period was extended with incandescent lamps to give a 16-hour photoperiod. The relative humidity of the air was not controlled and varied between 55-80 per cent.

Cultivation and preparation of plant material

Seed was fumigated with methyl bromide and placed in moist cold storage for the standard period. It was then sown in peat jiffy-pots, one seed per pot, and germinated in a phytotron glasshouse at 24/19°C. When the seedlings were approximately two weeks old, they were transplanted into 7" polystyrene pots, four seedlings being planted in each of 60 pots. Plants were watered in the morning with a modified Hoagland's solution in which the P level was half of normal, and in the afternoon with deionised water.

Eight weeks after germination, the height of each seedling was measured and, based on this assessment, the most uniform pair of diametrically opposite seedlings in each of 48 pots was selected for the experiment. At monthly intervals during the experiment, the base of the terminal rosette of each seedling was marked with indian ink - these marks were later used to determine the age of foliage on specific sections of stem.

Method of imposing shade treatments

Shade screens were constructed from Nos. SH-603 and SH-607 'Sarlon' shade cloth. Preliminary tests had shown that these cloths cast 46 per cent and 74 per cent shade respectively (i.e., they allowed 54 per cent and 26 per cent respectively of incident light to penetrate). The tests also showed that the level of shade cast is constant irrespective of the intensity of the light source, whether it be natural or artificial. Additional tests, using a Shimadzu photoelectric spectrophotometer, were conducted to determine whether

the shade cloths transmitted light uniformly over the wave length band 400-1000 mμ. Transmission was constant over the full range with the 46 per cent cloth. In the case of the 74 per cent cloth, however, it was constant from 400-700 mμ, but increased gradually in the range 700-1000 mμ. At 800, 900, and 1000 mμ, transmission was 8 per cent, 18 per cent, and 24 per cent higher respectively than at 700 mμ. A search of the literature has failed to locate any data indicating whether or not the observed increase in transmission at the longer wavelengths is likely to have any formative effects on plant growth.

Design of experiment

A factorial design was used. The level of experimental error was reduced by stratifying the pots into blocks on a height basis, i.e., with four pots per treatment, the pots with the tallest seedlings were placed in Block 1, those with the next tallest in Block 2, and so on until four blocks were filled. Treatments within blocks were applied at random, the two seedlings in each pot receiving the same treatment. The experiment involved twelve treatments comprising all combinations of four temperature regimes and three shade regimes, viz.:

Shade regime (%)	Temperature regime (°C)			
	15/10*	21/16	27/22	33/28
0	A	D	G	J
46	B	E	H	K
74	C	F	I	L

* 15/10 signifies a day temperature of 15°C and a night temperature of 10°C

Procedure with gas exchange measurements

Measurements of CO_2- and H_2O-vapour exchange were made when stock was 8-9 months old. Each seedling was examined only at the photo-temperature at which it was grown, e.g., in the 33/28°C regime, examination was at 33°C; in the 27/22°C regime, it was at 27°C; etc. Measurements were made on one seedling selected at random from each of Blocks 1-4. Since light response curves of photosynthesis could be determined for only two seedlings on any one day, measurements on the seedlings from the twelve treatments of Block 1 were conducted in the first week, those on the seedlings of Block 2 in the second week, and so on. This procedure enabled differences between blocks due to age effects (if any) to be partly eliminated in the analysis of variance.

As the gas exchange capacity of foliage varies with age (Freeland, 1952; Forsyth and Hall, 1965), the precaution was taken of using fully expanded attached needles of the same chronological age ($\pm$ 1 week in any one block). This necessitated that the tops of the plants be severed just above the upper level of insertion of the foliage on which gas exchange was to be determined, to eliminate shading by the apical shoot. It was assumed that severance would have little effect on the rate of exchange of foliage in the period of three hours after severance during which measurements were made (see Study 4). The cut surface of the stem was smeared with vaseline and covered with aluminium foil to eliminate water loss.

Respiration rates, which are expressed per unit volume of foliage, do not make allowance for the respiration of the length of stem to which the foliage was attached. Thus, the data presented are somewhat inflated. Rates of net photosynthesis and transpiration are also affected, but to a lesser extent. Steps were taken to minimise the effect that this source of error might have on differences between treatments by ensuring that the quantity of foliage used in the gas exchange measurements was approximately the same in all treatments. This procedure ensured that the volume of stem enclosed in the assimilation chamber was held reasonably constant - it also minimised differences between treatments in the self shading of foliage.

RESULTS

Tables 4 and 5 summarise the effects of temperature and shading during growth on some morphological and physiological features and on the dry matter production of P. radiata seedlings at 8 to 9 months of age. Full experimental data are presented in Appendix 5 (Tables A4-A38). The effects of treatment in a majority of cases are highly significant. The temperature x shading interaction in most cases is non-significant. Some plant responses are related directly to a shading or temperature increase, others are related inversely, while others again either tend to attain a maximum at some intermediate shading or temperature level or are unrelated to one or both environmental factors.

Optimum dry matter production in the P. radiata seedlings occurred under full sun and moderate

TABLE 4: Summary of the effects of temperature and shading during growth on some morphological features of P. radiata seedlings at 8 to 9 months of age. The experimental data are tabulated in Appendix 5.

Category	Feature	Temperature effect		Shading effect		T x S Interaction	Table no. (Appendix 5)
		Effect	Comments	Effect	Comments		
Needle and fascicle morphology	Needle length	+***	- but depression at 33/28°C under high shade	NS	----	NS	A4
	Needle thickness	-**	Most marked under full sun conditions	-***	----	NS	A5
	Length/thickness ratio	+***	ditto	+*	No effect at 33/28°C	NS	A6
	Surface area (individual needle)	*	Peaks at 21/16°C - 27/22°C	-**	----	NS	A7
	Fascicle sheath length	-***	----	*	Tends to peak under moderate shade	NS	A8
	Foliar angle	+***	----	+*	No effect at 15/10°C	**	A9
	No. stomatal rows/ needle	+***	----	-***	----	NS	A10
	Stomatal rows/mm of needle thickness	+***	----	NS	----	NS	A11
	No. of 3-needled fascicles	+***	Most marked with increase from 15/10°C to 21/16°C	NS	----	**	A12
	No. of 4-needled fascicles	-***	ditto	NS	----	*	A13
Plant morphology	Stem height	***	Peaks at 21/16°C under shade and 21/16°C - 27/22°C under full sun	***	Peaks under moderate shade	***	A14
	Stem basal diameter	***	ditto	-***	----	NS	A15
	Height/diameter ratio	+***	----	+***	----	***	A16
	Fascicles/unit length of stem	-***	Tends to peak at 21/16°C under shade	-***	----	NS	A17
	No. of branches	NS	----	-***	----	NS	A18
	Ratio $\frac{\text{Branch O.D.Wt.}}{\text{Stem O.D.Wt.}}$	-***	----	-***	----	NS	A19
	Stockiness	-***	Tends to peak at 21/16°C	-***	----	NS	A20

\+ Indicates an effect directly related to shading or temperature increase

\- Indicates an effect inversely related to shading or temperature increase

*, ** and *** Indicate significance of the treatment or interaction effect at the 5 per cent, 1 per cent, and 0.1 per cent levels respectively

NS Indicates non-significance

TABLE 5: Summary of the effects of temperature and shading during growth on some physiological features and on the dry matter production of P. radiata seedlings at 8 to 9 months of age. The experimental data are tabulated in Appendix 5.

Category	Feature	Temperature effect		Shading effect		T x S Interaction	Table no. (Appendix 5)
		Effect	Comments	Effect	Comments		
Needle physiology	[Ca]	+*	----	+***	----	NS	A21
	[Cb]	+***	----	+***	----	NS	A22
	[Ca+b]	+***	----	+***	----	NS	A23
	[Carotenoids]	+*	----	+***	----	NS	A24
	Ca/Cb	-***	----	-**	No effect at 27/22°C and 33/28°C	NS	A25
	Ca+b/Carotenoids	NS	----	NS	----	NS	A26
	Leaf moisture content	NS	----	+**	----	NS	A27
	Dark respiration	+***	Tends to a minimum at 21/16°C	-***	----	NS	A28
	Light compensation point	+***	----	-***	----	NS	A29
	Initial slope of light response curve	-***	----	+***	----	*	A30
	Net P 500 ft.c.	-***	----	+***	----	NS	A32
	Net P 1500 ft.c.	-***	----	+***	----	*	A32
	Net P 2500 ft.c.	-***	----	+***	----	**	A32
	Net P 3500 ft.c.	-***	----	+***	----	***	A32
	P3500/R ratio	-***	----	+***	----	*	A31
	Transpiration 500 ft.c.	NS	----	+***	----	NS	A33
	Transpiration 1500 ft.c.	+***	----	+***	----	NS	A33
	Transpiration 2500 ft.c.	+***	----	+***	----	NS	A33
	Transpiration 3500 ft.c.	+***	----	+***	----	*	A33
	P/T ratio (3500 ft.c.)	-***	----	-***	----	NS	A34
Dry matter production	O.D.Wt. shoot	***	Peaks at 21/16°C	-***	----	*	A35
	O.D.Wt. root	***	ditto	-***	----	**	A36
	O.D.Wt. whole plant	***	ditto	-***	----	*	A37
	Root/shoot ratio	-***	Little effect over range 15/10°C - 27/22°C	-***	----	NS	A38

\+ Indicates an effect directly related to shading or temperature increase

\- Indicates an effect inversely related to shading or temperature increase

*, **, and *** Indicate significance of the treatment or interaction effect at the 5 per cent, 1 per cent, and 0.1 per cent levels respectively

NS Indicates non-significance

temperature conditions. The production of shoots, roots, and whole plants was highest under the 21/16°C temperature regime irrespective of the light conditions imposed during growth (Appendix 5, Tables A35-A37).

Measurements by planimeter on the cross-sections of representative needle sections (Plates 1-4) indicate that, irrespective of growing conditions, the various leaf tissues form a constant proportion of the total leaf cross-sectional area, viz., epidermal tissue, .16 ± .01; mesophyll tissue, .52 ± .01; vascular tissue, .32 ± .01. Because of limited time, no other quantitative measurements of leaf anatomical structure were made. Rather, sections were examined qualitatively, aiming solely at detecting obvious differences in structure which might affect the capacity of leaves to photosynthesise, e.g., differences in the development and arrangement of palisade and sponge mesophyll tissue, in the abundance of intercellular spaces, and in the depth of the stomatal chamber (see Appendix 6). Each of these could affect leaf diffusive resistance. However, no obvious differences are apparent (Plates 1-4).

DISCUSSION

Leaf and whole plant morphology

With the exception of the effect of temperature on the grouping of needles in a fascicle, the responses observed in leaf and whole plant morphological features to the light and temperature conditions imposed during growth (Table 4) are similar to responses observed in other species. One interesting aspect is the large

PLATE 1 Transverse sections of needles of P. radiata seedlings grown under a 15/10°C regime.

Upper : 0% shading

Middle : 46% shading

Lower : 74% shading

Sections photographed under low power at a magnification of 50X.

Each minor scale division represents 0.01 mm.

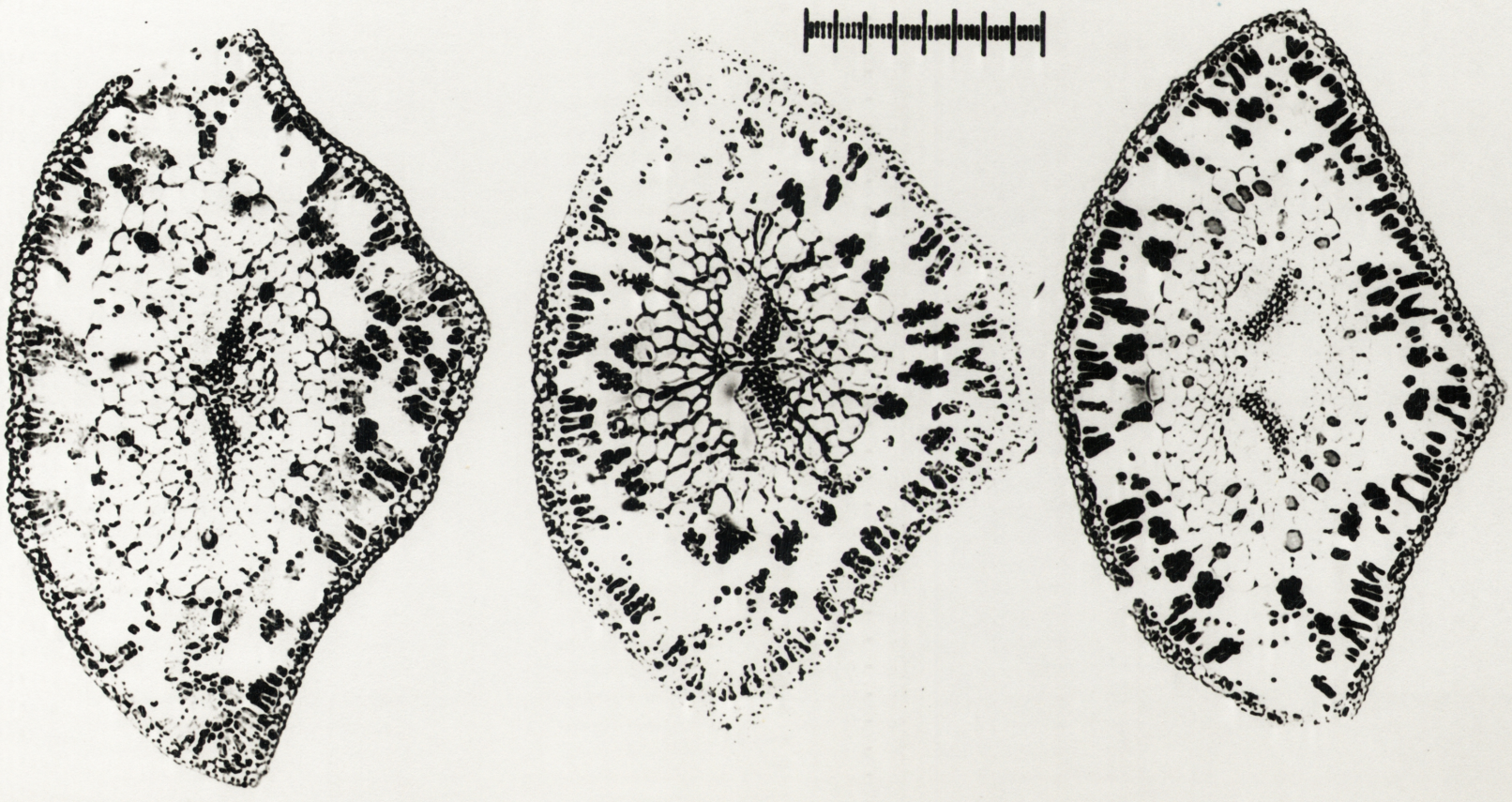


PLATE 2 Transverse sections of needles of P. radiata seedlings grown under a 21/16°C regime.

Upper : 0% shading

Middle : 46% shading

Lower : 74% shading

Sections photographed under low power at a magnification of 50X.

Each minor scale division represents 0.01 mm.

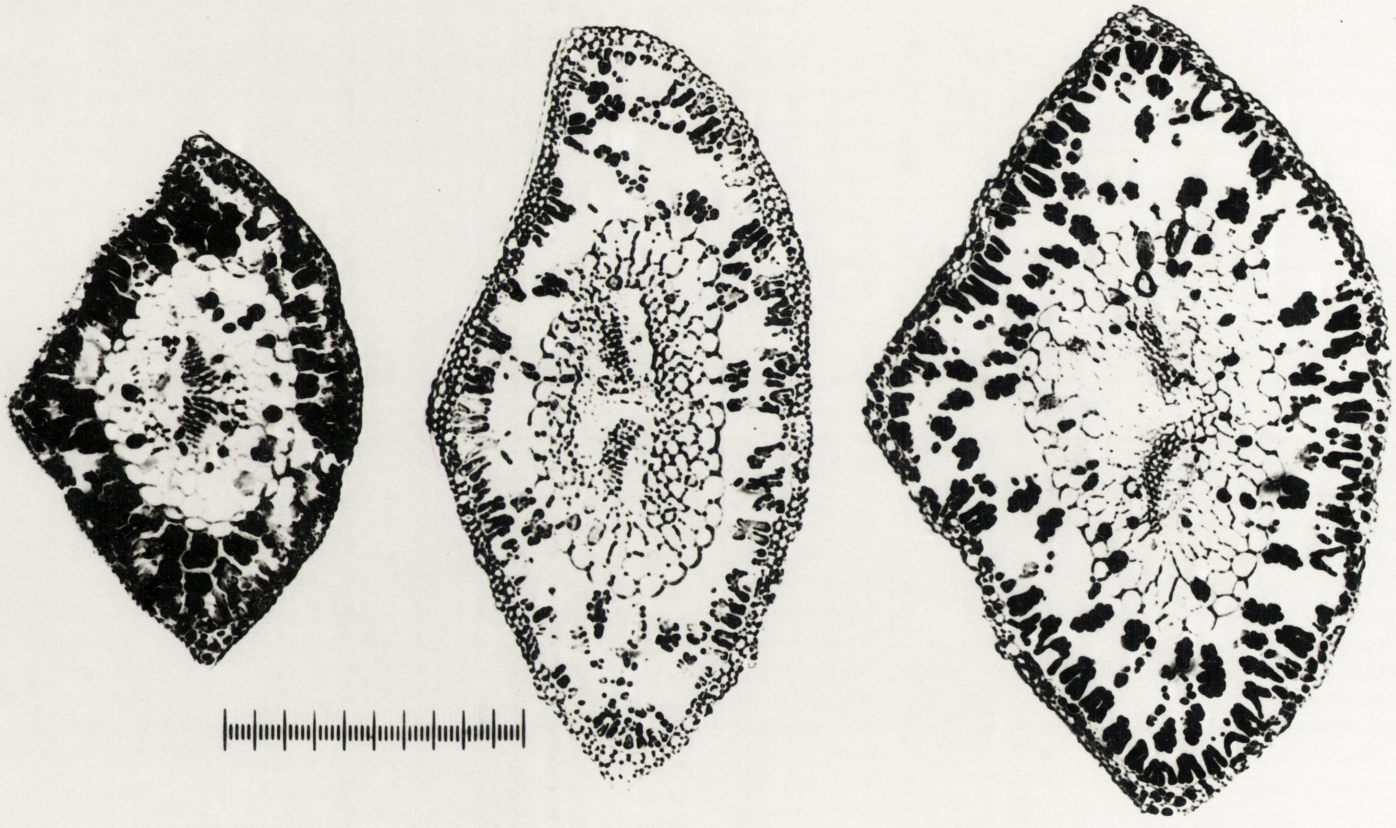


PLATE 3 Transverse sections of needles of P. radiata seedlings grown under a 27/22°C regime.

Upper : 0% shading

Middle : 46% shading

Lower : 74% shading

Sections photographed under low power at a magnification of 50X.

Each minor scale division represents 0.01 mm.

PLATE 4 Transverse sections of needles of P. radiata seedlings grown under a 33/28°C regime.

Upper : 0% shading

Middle : 46% shading

Lower : 74% shading

Sections photographed under low power at a magnification of 50X.

Each minor scale division represents 0.01 mm.

proportion of these responses which might be useful to the plant in the particular environment in which it is growing. For example, the morphology of seedlings under shade was characterised by light branching, high leaf angles, widely spaced leaves on the stem, and, under 46 per cent shade, by stem etiolation (Appendix 5, Tables A18/A19, A9, A17, and A14 respectively). In addition, pigment concentration was high (Appendix 5, Tables A23, A24). These features collectively would increase the display of foliage to light and the capacity of leaves to absorb light. The high photosynthetic capacity of needles on shade grown plants (Appendix 5, Table A32) is probably evidence that this was so. Such responses might thus be considered to be of a compensatory nature. These compensatory responses are discussed further in the general discussion.

The basic factors determining the number of needles per fascicle in the genus Pinus are unknown. The differences between species are probably largely under genetic control, whereas those between individuals of the same species, and especially within the one individual, are probably environmentally induced. The results of this study confirm temperature as a determinant (see Shaw, 1914), the trend in the P. radiata seedlings examined being for 3-needled fascicles to predominate at moderate temperatures and above (21/16°C+) and 4-needled fascicles at 15/10°C (Appendix 5, Tables A12, A13). Some 2- and 5-needled fascicles were also recorded - these occurred exclusively

at 33/28°C and 15/10°C respectively, thus fitting the general pattern established for the intermediate order fascicles.

Haller (1965) and Squillance (1966) consider that the number of needles per fascicle may have adaptive value. Squillance hypothesises that higher order fascicles may be more efficient photosynthetically than lower order fascicles. He bases his hypothesis on the assumption that the former have more surface area available for the interception of light and exchange of gases. This assumption may not be valid if the increase in needles per fascicle is accompanied by a decrease in needle size (length and/or thickness). However, measurements made on 3- and 4-needled fascicles in the 15/10°C full sun treatment of this experiment indicate a negligible effect of fascicle order on both needle length and thickness, viz.:

Fascicle order	Needle length - cm	Needle thickness - mm
3-needled	12.0 ± 0.2	0.76 ± 0.01
4-needled	12.3 ± 0.2	0.75 ± 0.01

Assuming that the cross-sectional shape of a fascicle is circular, the needles in the fascicle are of equal size, and their inner faces approximate the radius (R) of the fascicle cross-section (Appendix 1), then the ratio of the surface area of needles in 4- as compared to 3-needled fascicles, when needle length and thickness are constant, is:

$$\frac{4\left(\frac{2\pi R}{4} + 2R\right)}{3\left(\frac{2\pi R}{3} + 2R\right)} = \frac{\pi + 4}{\pi + 3} = 1.163$$

This ratio represents a surface area advantage for 4-needled fascicles which could affect their photosynthetic efficiency. It may partly explain the high rates of net photosynthesis of stock raised under the 15/10°C temperature regime (Appendix 5, Table A32). In this regime, the incidence of 4- and 5-needled fascicles in the various shade treatments was high, viz., 44 per cent to 86 per cent (Appendix 5, Table A13).

Leaf physiology

Leaf pigments: The changes induced in the concentration of chlorophyll and carotenoids in the leaf, by varying the light intensity and temperature conditions during growth, appear to have been closely in phase because the chlorophyll/carotenoid ratio was not significantly affected by treatment (Appendix 5, Table A26). The concentration of each pigment over the range of treatments investigated was maximum under the high shade/high temperature regimes (74 per cent shade, and 27/22°C - 33/28°C: Appendix 5, Tables A23-A24), presumably because the balance between pigment synthesis and decomposition in these regimes was most favourable for pigment accumulation. The trends, overall, may be interpreted as examples of the well known response of pigment concentration to light intensity and temperature.

The range of Ca/Cb values observed (2.1-2.9) falls within the range commonly found in plants (2.0-3.5). The depression in the ratio under low light conditions is a characteristic plant response (cf. Rabinowitch, 1945; Tieszen and Bonde, 1967), but its significance is

not clear since the role of chlorophyll-b in the physiological functions of a plant is not fully understood. However, variation in the Ca/Cb ratio can cause a minor change in the absorption spectrum of leaves (Rabinowitch, op. cit.). Thus, the changes observed might indicate chromatic adjustments by the plant enabling it to be more efficient in the utilisation of light in the prevailing light climate.

CO_2- and H_2O-vapour exchange: The comparatively low rate of dark respiration of foliage of shade grown seedlings (Appendix 5, Table A28) is a feature well known in plants. It is thought to be due to low levels of enzymatic activity and proportionately smaller amounts of respiring tissue in shaded stock (Grime, 1966). Changes in the proportion of respiring tissue are unlikely to be causal in this instance because results already presented indicate that the relative proportions of the epidermal, mesophyll, and vascular tissues in the leaf were unaffected by the light and temperature conditions imposed during growth.

The tendency for the dark respiration rate of P. radiata seedlings grown at 15/10°C to be higher than that of seedlings grown at 21/16°C was also observed by Rook (1966, 1969). Such a trend is generally attributed to the higher level of soluble sugars in cold adapted plants (Warren Wilson, 1966). Presumably growth is inhibited more than photosynthesis in cold adapted seedlings enabling a surplus of photosynthate in the form of soluble sugars to accumulate. The reason behind the higher respiratory rates, however, needs further investigation.

A notable feature of the physiology of seedlings grown in full sunlight is the comparatively low initial slope of the light response curves of photosynthesis (Appendix 5, Table A30). This indicates a lower capacity of the photochemical process in sun grown than in shade grown plants. As the initial slopes correlate positively with concentration of chlorophyll (Table 6), we may interpret the reduction in the capacity of the photochemical reaction in sun grown plants as being due to an impairment of the light absorbing system.

The capacity of the photochemical process in seedlings grown under uniform conditions was shown earlier to be identical when CO_2-uptake was measured over the temperature range 11^{o}-29^{o}C. Nevertheless, the temperature regime under which seedlings are grown has a significant effect on the slope of the light response curve (Appendix 5, Table A30). It is probable that the discrepancy between the two situations is due to the leaf characteristics of the seedlings. In the first case they were all similar, whereas those in the latter situation differed substantially in morphology and pigment content and, presumably, in their capacity to absorb light.

The decrease in the compensation point for light with decrease in either the light or temperature climate for growth (Appendix 5, Table A29) is probably related to the concomitant decrease in the rate of dark respiration and increase in the capacity of the photochemical partial process of photosynthesis. A low compensation point, which is almost invariably found in

TABLE 6 Tests of significance of the correlation coefficients of the regression equations, relating the rate of net photosynthesis at various levels of illumination, and the slope of the light response curve of photosynthesis, to the concentration of chlorophyll in seedlings raised in a controlled environment under a range of shading and temperature regimes

Variable (X) *	Variable (Y) **	Regression equation	Correlation coefficient	Significance level of correlation coefficient
[Ca+b]	Net P at 500 ft.c.	Y = -0.1074 + 0.0751X	0.59	5%
"	Net P at 1500 ft.c.	Y = 0.2396 + 0.1701X	0.55	5%
"	Net P at 2500 ft.c.	Y = 0.5475 + 0.1806X	0.49	NS
"	Net P at 3500 ft.c.	Y = 0.6504 + 0.1833X	0.45	NS
"	Slope of light response curve	Y = 0.8486 + 0.2350X	0.62	5%
[Ca]	Net P at 500 ft.c.	Y = -0.1940 + 0.1293X	0.67	1%
"	Net P at 1500 ft.c.	Y = 0.0196 + 0.2987X	0.64	5%
"	Net P at 2500 ft.c.	Y = 0.2827 + 0.3250X	0.58	5%
"	Net P at 3500 ft.c.	Y = 0.3591 + 0.3356X	0.54	5%
"	Slope of light response curve	Y = 0.5957 + 0.3999X	0.70	1%

* Concentration of chlorophyll expressed in mg/g dry weight

** Net photosynthesis expressed as mg CO_2/c.c./hr

NS Non-significant

a shade adapted plant, is of value to a plant in a low light situation and is a component of shade tolerance (Wareing, 1966). Net photosynthesis at all light intensities increased with increase in the shade level at which seedlings were grown (Appendix 5, Table A32). This is a commonly observed phenomenon (Pisek and Tranquillini, 1954; Jarvis, 1964). It occurs almost invariably at low light intensities because of the high capacity of the photochemical process in shade adapted plants; and might be anticipated at higher light intensities in the study material because of the positive correlation established between pigment content and the rate of net photosynthesis (Table 6). This correlation is highest when chlorophyll-a alone is considered (cf. Šesták, 1966). Other factors which might serve to increase the net photosynthetic rate of the shade adapted seedlings are the low dark respiration rate (Appendix 5, Table A28) and, possibly, the higher proportion of functional primary leaves which the seedlings were observed to carry. Similar leaves in P. taeda are very efficient photosynthetically (Bormann, 1956, 1958). Differences in stomatal density and in the number of needles per fascicle are not involved as these parameters were unaffected by shading (Appendix 5, Tables A11-A13). The angle of insertion of foliage is ruled out as a contributing factor as it was unaffected by shading at 15/10°C and 33/28°C (Appendix 5, Table A9) and yet shading at these temperatures led to a substantial increase in photosynthetic capacity (Appendix 5, Table A32).

The trend towards a depression, at all shade levels, of the rate of net P with increase in temperature, is in contrast to the trend of transpiration which showed an overall rise (Appendix 5, Tables A32-A33). The latter does not necessarily imply that stomates remained fully open as the potential for water vapour loss at the higher temperatures probably increased greatly.

The very low rates of net P observed in the stock raised under full sunlight at 27/22°C are difficult to explain as dry matter production in this treatment was high (Appendix 5, Table A37). The effect was real, however, as it was observed in each of the four replicates examined. Water stress is the probable explanation because the rate of transpiration of the stock was also strongly depressed (Appendix 5, Table A33). This suggests that stomatal control of water loss was operating.

The wide range in the rates of net P at light intensities approaching saturation (Figure 6) is evidence of the substantial effects of the light and temperature regimes during growth on those leaf morphological and physiological features which affect the efficiency of the photosynthetic process. The significance of the increasing temperature x shading interaction on the rate of net photosynthesis as illumination is increased from 500 to 3500 ft.c. (Table 5) is not clear. Possibly it is an indirect result of the fact that temperature does not affect the rate of photosynthesis at low light intensities when the photochemical reaction is light limited.

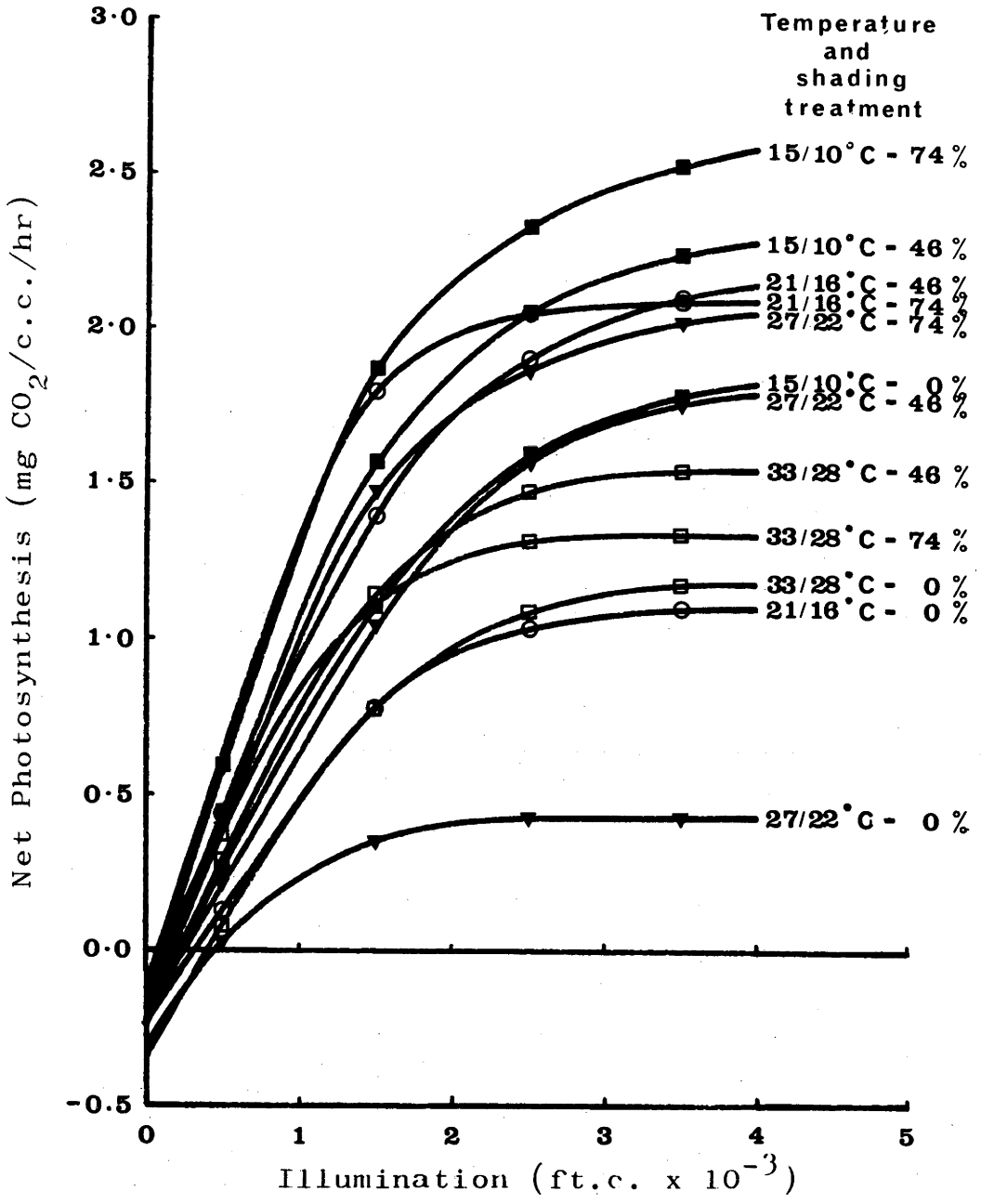


FIGURE 6 : Light response curves of net photosynthesis for 8 to 9-month-old P. radiata seedlings grown in a controlled environment under a range of temperature and shading regimes. Response curves were determined at the day temperature, e.g., in the 15/10°C regime, at 15°C; in the 21/16°C regime, at 21°C; etc.

The higher rate of transpiration observed in the shade adapted than in the sun adapted seedlings (Appendix 5, Table A33) is contrary to what is usually found (Daubenmire, 1947). The reversal of trend may be explained if the stock raised under full sun conditions was suffering the effects of water stress at the time of gas analysis. While there is no direct evidence that this was so, some indirect evidence suggests, as mentioned previously, that the stock raised at 27/22°C under full sun conditions was water stressed. Part of the explanation for the trend observed may lie in the use of volume as the basis for expressing the rate of exchange. The rate of transpiration in sun adapted plants, for example, is greater per unit of leaf area than per unit of leaf weight (Baker, 1950). Much the same relationship should hold for volume. For a satisfactory explanation of the phenomenon, one needs information on the magnitude of the cuticular component of transpiration and of the various leaf diffusive resistances in the water vapour diffusion pathway in leaves from the various treatments. This information is currently not available.

The effect of temperature on the rate of transpiration of foliage is marked particularly in stock raised under shade (Appendix 5, Table A33). The increased capacity for exchange with rise in temperature is normal (Kozlowski and Keller, 1966) and is partly due to a steepening of the diffusion gradient between the evaporating sites in the leaf and the external atmosphere. The extent to which variation in

the leaf diffusive resistances contributes to the trend is not known.

Dry matter production

The marked fall in dry matter production under high shade at high temperatures (Appendix 5, Tables A35-A37) is to be expected because while high temperatures reduce yields per se, they become increasingly critical for growth when light is limiting (Stansel, 1966). Under these conditions, total photosynthesis is little affected by temperature increase while respiration is accelerated (Figure 4). This leads to a reduction either in the amount of stored foods or in the rate of their accumulation.

It seems anomalous that seedlings grown in full sunlight should produce most dry matter (Appendix 5, Table A37) and yet exhibit low capacities of net photosynthesis per unit of leaf volume (Appendix 5, Table A32). The yield of a plant, however, is determined by the total amount of light intercepted by its foliage as well as by the photosynthetic capacity of the individual leaf. In this respect, measurements made on seedlings grown under full sun conditions at 15/10°C and 27/22°C revealed that they carried 3.6 and 5.8 times respectively the dry weight of foliage of seedlings raised under 74% shade. These ratios are roughly in agreement with the ratios of shoot dry weight (Appendix 5, Table A35). Thus, if allowance is made for the greater mass of foliage carried by sun grown plants, it might be possible to reconcile low capacities of net photosynthesis per unit of leaf with high levels of dry matter production per plant. No

attempt has been made to reconcile the data in this instance as the photosynthetic studies involved isolated measurements taken at a particular stage of seedling development. They bear little relation to the integrated yields of daily net photosynthesis which would be required for a valid comparison with seedling dry matter production.

The changes observed in the root/shoot ratio following manipulation of the environmental conditions during growth (Appendix 5, Table A38) do not necessarily imply that the relative growth rates of the roots and shoots were altered. Plotting the logarithms of the root and shoot dry weights against each other establishes a linear relationship (Figure 7). This indicates that the roots and shoots of seedlings in all treatments had the same relative growth rate, and that the change in the R/S ratio, due to variation in the shading and temperature regimes during growth, was merely a correlated result of altering plant weight (Wareing, P.F. - in Bormann, 1958, p.208; Ledig and Perry, 1966). Changes observed in the R/S ratios of plants are frequently misinterpreted because of the failure to recognise that the ratio is largely a function of plant size.

While no quantitative measurements other than dry weight were made on the root systems of seedlings, temperature was observed to affect the external morphology of roots quite markedly. The roots of seedlings grown under the 15/10°C regime, for instance, were large and infrequently branched, and varied from white to light tan in colour; whereas those grown at

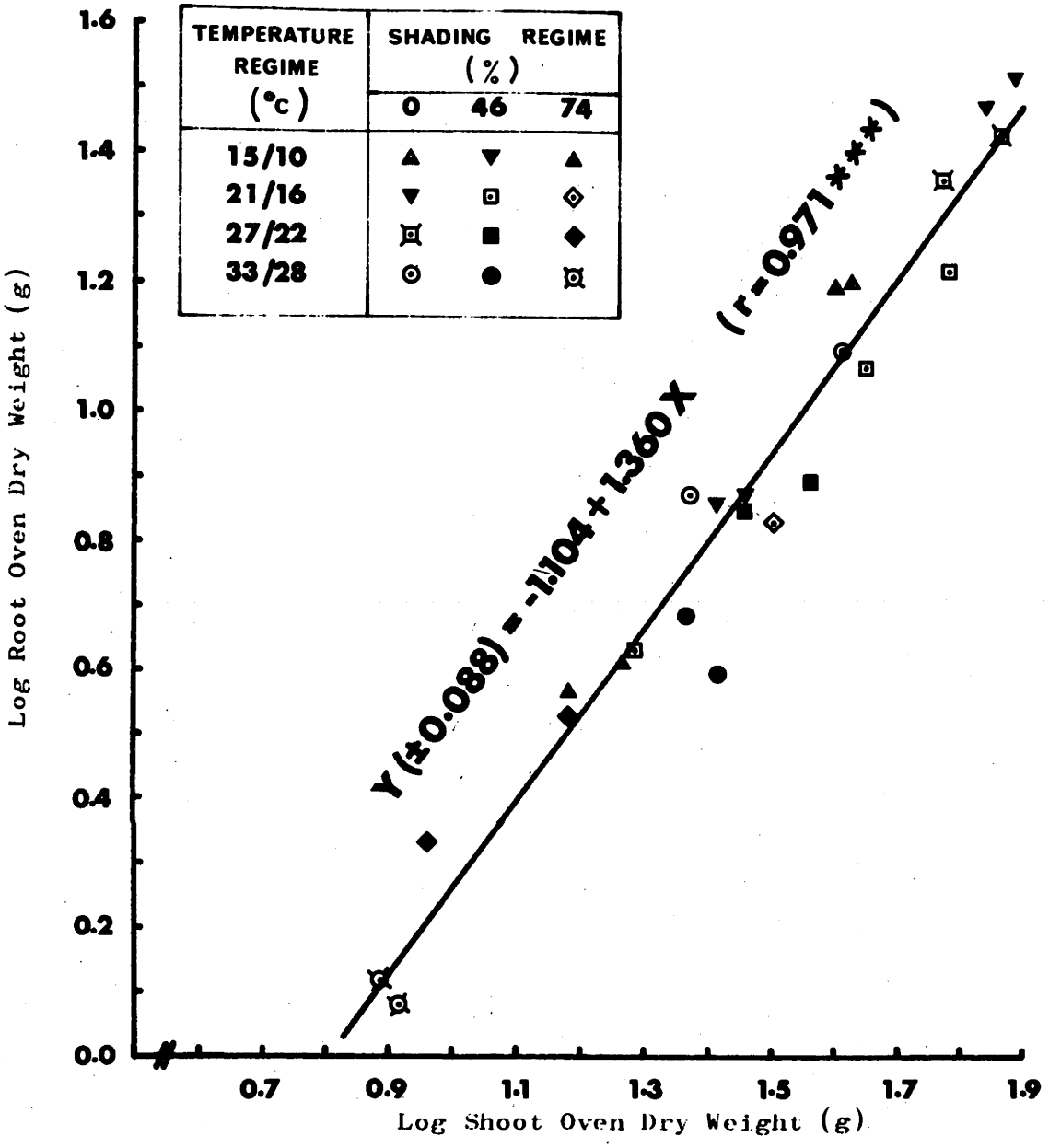


FIGURE 7 : Regression of log root oven dry weight on log shoot oven dry weight for 8-month-old P. radiata seedlings grown under a range of temperature and shading regimes.

33/28°C were fewer, thinner, extensively branched, and dark brown in colour with small light brown tips (cf. Hellmers, 1966; Nielsen and Humphries, 1966).

STUDY 3

EFFECT OF SOIL MOISTURE DEFICIT AND EXCESS ON THE GAS EXCHANGE CAPACITY AND GROWTH OF SEEDLINGS

The objective of the study, which was confined to seedling material, was to follow the pattern of net photosynthesis both during repetitive drought cycles and during a flood cycle, and to assess the effect of various soil moisture regimes on seedling height growth and dry matter production.

MATERIALS AND METHODS

Three experiments were established to meet the study objective. Experiments 1 and 2 examined the pattern of gas exchange in droughted and flooded seedlings respectively, while the growth response of seedlings to various soil moisture regimes was assessed in Experiment 3.

Stock was raised in the standard potting mix. The moisture content of this soil at suctions of 100 cm of water and 15 atm was 15.2 per cent and 4.4 per cent respectively. These moisture contents were used as rough guides to the 'field capacity' and 'permanent wilting percentage'.

The materials and methods specific to each experiment were as follows:

Experiment 1

Two healthy 2-year-old seedlings (A and B) raised in 9" polystyrene pots were used. Soil moisture was monitored with matched porous conductivity blocks implanted in the soil at both the 1/3 and 2/3 levels of soil depth. Plant water status was assessed by the relative water content technique (Appendix 4). As preliminary tests had indicated that the determination of the RWC of a uniform batch of foliage was highly reproducible (the range generally being less than $\pm$ 0.5 per cent of the average value) each determination was confined to two replicates. Even this degree of sampling involved, over a period of 1-2 months, the destruction of a significant proportion of needles on seedlings A and B - 10 per cent and 30 per cent respectively of the stems of each were left bare of foliage.

Because sampling for RWC had to be confined to foliage not involved in the determination of CO_2-exchange, the procedure adopted was to sample the foliage immediately below the assimilation chamber. With seedling A, this involved sampling fascicles at random from a pre-designated 15 cm (10 per cent) length of stem. In seedling B, three such sections were necessary, one section being used for both the first and second drying cycles and the other two for the third and fourth cycles respectively. As a pilot test had indicated that the RWC of foliage at a given insertion level on P. radiata seedlings differs from that of foliage at other levels, an experiment was established to assess the magnitude of differences.

This enabled the RWC of foliage at one level to be estimated by applying a correction to the RWC determined at another level. The experiment is described below:

Four potted 11-month-old P. radiata seedlings were well watered for several days, after which the RWC of foliage at the 10, 30, 50, 70, and 90 per cent insertion levels (per cent of total stem height) on each seedling was determined. The soil moisture content was measured gravimetrically at the same time, a $\frac{1}{2}$" bore auger being used to extract samples. The plants were then allowed to dry out by evapotranspiration for five days, and then for a further three days. At the end of each of these periods, the RWC of foliage and soil moisture content were determined. Results are presented in Table 7.

TABLE 7 Effect of level of insertion of fascicles on the relative water content (%) of 11-month-old P. radiata seedlings at each of three soil moisture levels

Soil moisture content (% dry weight)	Insertion level of fascicles (% of total height)					Mean
	10	30	50	70	90	
			(±1.3)			(±0.6)
15 (≈FC)	80.4	83.1	87.6	91.9	93.4	87.3
7	83.1	81.5	87.0	90.9	92.4	87.0
4 (≈PWP)	67.6	67.5	74.4	75.1	71.9	71.3
Mean (±0.8)	77.0	77.3	83.0	86.0	85.9	81.8
	L.S.D. 5% 3.8		L.S.D. 1% 5.1		L.S.D. .1% 6.6	

The data indicate a highly significant effect ($p < .001$) of both the level of insertion of fascicles and the soil moisture content on the RWC of needles. The values of the L.S.D.s indicate that there is a significant difference between the RWCs of foliage at the 30 per cent and 50 per cent insertion levels. The implication for the drought experiment is that the RWC data to be presented for seedling A are likely to underestimate the actual RWC values by approximately 2-3 percentage units (Figure 10); whereas those presented for seedling B are likely to underestimate the actual values by approximately 2-3 units on the first and second drying cycles and by approximately 5-8 units on the third and fourth drying cycles (Figure 11). A check made on seedling B at the end of the fourth cycle confirmed this, i.e., the RWC of needles within the plant chamber at the 50 per cent insertion level was 72 per cent as against 64 per cent RWC at the 20 per cent insertion level.

A complication arose in the measurement of the needle RWC nearing the completion of experimentation on both seedlings A and B. Severe water stress caused the needle tips to desiccate and die. Tests conducted on duplicate samples, in which the dead tips were left on one sample and removed from the other, indicated that up to 15 per cent of the total needle length (the highest percentage desiccation investigated) could die from the tip without affecting the determination of the RWC. Once desiccation exceeded 15 per cent, the measurement of RWC was discontinued.

Seedlings were prepared for gas analysis by severing their tops and smearing the wounds with vaseline prior to covering the cut surfaces with aluminium foil (to minimise water loss from these surfaces). The foliage enclosed in the assimilation chamber was 8-9 months old and was fully expanded. Test conditions included a constant air flow rate and relative humidity, an illumination of 2500 ± 100 ft.c., and a temperature of 25 ± 1°C. When gas analysis was not being conducted, the seedlings were held in the growth cabinet under the same light and temperature conditions, and with a 12/12 photoperiod and a 20°C nycotemperature. As stomatal closure is thought to occur earlier each day during a developing drought (Kozlowski, 1964; Philpot, 1965), net photosynthesis, transpiration, and the RWC were always measured at a standard time, viz., 1000 hours, and dark respiration at 1100 hours. As the same foliage was used throughout the trial, exchange capacities are expressed as a percentage of the maximum rate observed per shoot .

The treatments to which seedlings A and B were subjected were as follows:

Seedling A - gas exchange was first measured on 7/11/66 (Day 0). The plant was then given a light watering as the soil moisture content was low (approximately 7 per cent). Further watering was withheld until evapotranspiration had so dried the pot and plant that it was considered the seedling had little chance of recovery if rewatered. This stage was reached by Day 13. The plant was then rewatered.

Seedling B - gas exchange measurements commenced on 15/12/66 (Day 0). The plant was allowed to dry out by evapotranspiration over a series of four cycles, each drying cycle with the exception of the 4th being of longer duration than the previous one. The trial was terminated as soon as the rate of net photosynthesis in the 4th cycle fell to zero, i.e., on 3/2/67 (Day 50).

Experiment 2

A healthy 12-month-old potted seedling was transferred to a growth cabinet and acclimatised for one week under the following conditions: Illumination - 2500 ± 100 ft.c.; Phototemperature - 25 ± 1°C; Nycotemperature - 16 ± 1°C; Photoperiod - 12/12. The terminal 20 cm of the seedling shoot were then enclosed in the plant chamber, and the rates of net photosynthesis and transpiration were measured at 1020 hours on each of two consecutive days. Light and temperature conditions, etc., were the same as those to which the plants had been acclimatised. The pot containing the seedling was then placed in a bowl of tap water so that the surface of the pot was submerged to a depth of 1". The rates of net photosynthesis and transpiration were monitored continuously from the time of submergence until 1720 hours on the first day of submergence, and from approximately 0900-1700 hours on the second and third days. On the fourth day, a continuous measurement was made from 'lights on' at 0600 hours to 30 minutes after 'lights off' at 1800 hours, the purpose being to follow the diurnal pattern of net photosynthesis and transpiration in a plant under

flood conditions. In the following 3 weeks, net photosynthesis and transpiration were measured at 1200 hours at intervals of 1-3 days, and in the 4th to 6th weeks after submergence, at 1200 hours at intervals of approximately 1 week. At the end of the 6th week, as the foliage of the plant was severely chlorotic and the gas exchange was rapidly falling to negligible proportions, the flood conditions were removed and the pot was allowed to drain. Gas exchange was measured at 1200 hours at intervals of one week for the next two weeks, and was then discontinued. Daily inspections were made in the following weeks until it was obvious that the plant had survived.

During the first 16 days of submergence, the flood bath was re-charged with fresh tap water every four days, but as it appeared that the seedling had become somewhat adapted to the conditions imposed, the bath was allowed to stagnate. This was hastened by bubbling O_2-free nitrogen through the bath for periods of from 10-60 minutes daily from the 3rd to the 6th week, and by partially sealing the bath from the atmosphere using polythene sheeting. These treatments should have hastened the onset of anaerobic conditions (Kramer and Kozlowski, 1960).

Experiment 3

Seedlings were raised in seed trays under glasshouse conditions. When six weeks old, they were transplanted into tared, undrained, enamel pots. During the next $2\frac{1}{2}$ months, they were watered to weight thrice daily to hold the soil at 'field capacity'. Water loss by evaporation from the soil surface was

shown by measurement to be less than 0.5 g per pot per day. This was achieved by placing a disc of polythene sheeting on the soil surface and a reflecting surface of white-painted threeply (appropriately slotted to receive the seedling) on the top of the pot.

When the stock was four months old, the height from the level of the cotyledon to the base of the terminal rosette was measured on each seedling. Pots were stratified into blocks on the basis of this height assessment, the layout being a 5 X 3 randomised block. Treatments were as follows:

A - Rewatered to 'field capacity' (15.2 per cent soil M.C.) when the soil moisture content fell to 6.2 per cent, i.e., when approximately 80 per cent of the 'available' water had been removed.

B - Rewatered to FC when the soil moisture fell to 10.7 per cent, i.e., when approximately 40 per cent of the 'available' water had been removed.

C - Held at or near FC by watering to weight thrice daily.

D - Rewatered to 19.7 per cent soil moisture content when the moisture content fell to FC.

E - Rewatered to 24.4 per cent soil moisture content when the moisture content fell to FC.

The above method of subjecting plants to various degrees of soil moisture stress is widely practised (Dickson et al., 1965; Hosner et al., 1965; Cooper et al., 1966). Pots within a block were rotated daily to reduce the effects of gradients of temperature and light in the glasshouse environment.

Treatments D and E initially resulted in the soils being semi-saturated and saturated respectively, whereas Treatments A and B gave droughted and semi-droughted conditions respectively. Force of circumstances necessitated that the treatments be maintained for approximately one year. As the watering weights were not altered as the plants developed and grew, the increasing amount of water held within the plant system served to reduce progressively the amount of water held in the soil. Thus, the actual treatments to which the plants were subjected altered with time. As it was necessary to be able to specify the treatments imposed at the beginning and end of the experiment (soil moisture content could not be determined from auger borings because of the abundance of roots) an estimate of the amount of water held by the plant was necessary. Measurement of the amount in the shoot was straightforward. Direct measurement of the amount in the root was avoided because recovery of roots from soil involves washing with water which could alter the root moisture status. The problem was overcome by establishing a small experiment to determine the ratio of the moisture content of roots to that of shoots at several levels of plant water stress. The experiment is described briefly below:

Nine 5-month-old potted P. radiata seedlings of uniform size and development growing in washed perlite were selected. The seedlings were well watered for one week, and then three were harvested to determine the fresh and oven dry weights of roots and shoots. Determination of the root fresh weight was simple as gentle shaking of the root ball rapidly dislodged the perlite granules, i.e., washing with water was avoided. Watering was withheld from the remaining seedlings for four days, at which stage three more seedlings were harvested, and then for another four days after which the final three seedlings were harvested. Seedlings from the intermediate sampling were beginning to exhibit the first sign of water stress, viz., drooping terminal shoot, whereas those in the final sampling were exhibiting severe symptoms of stress. The rooting medium at the time of the first sampling was wet throughout, that at the second sampling was dry in the upper quarter of the pot and damp in the lower threequarters, while that at the final sampling was dry except for a narrow layer at the bottom of the pot which was slightly damp.

The data for the moisture content of roots and shoots at each sampling, and the relative moisture content of roots/shoots are illustrated in Figure 8. The most significant feature of the graph is that root moisture content is strongly depressed by treatment whereas shoot moisture content is largely unaffected. This may be evidence that roots are more susceptible to the effects of moisture stress than are shoots. Figure 8 also illustrates that the effect of

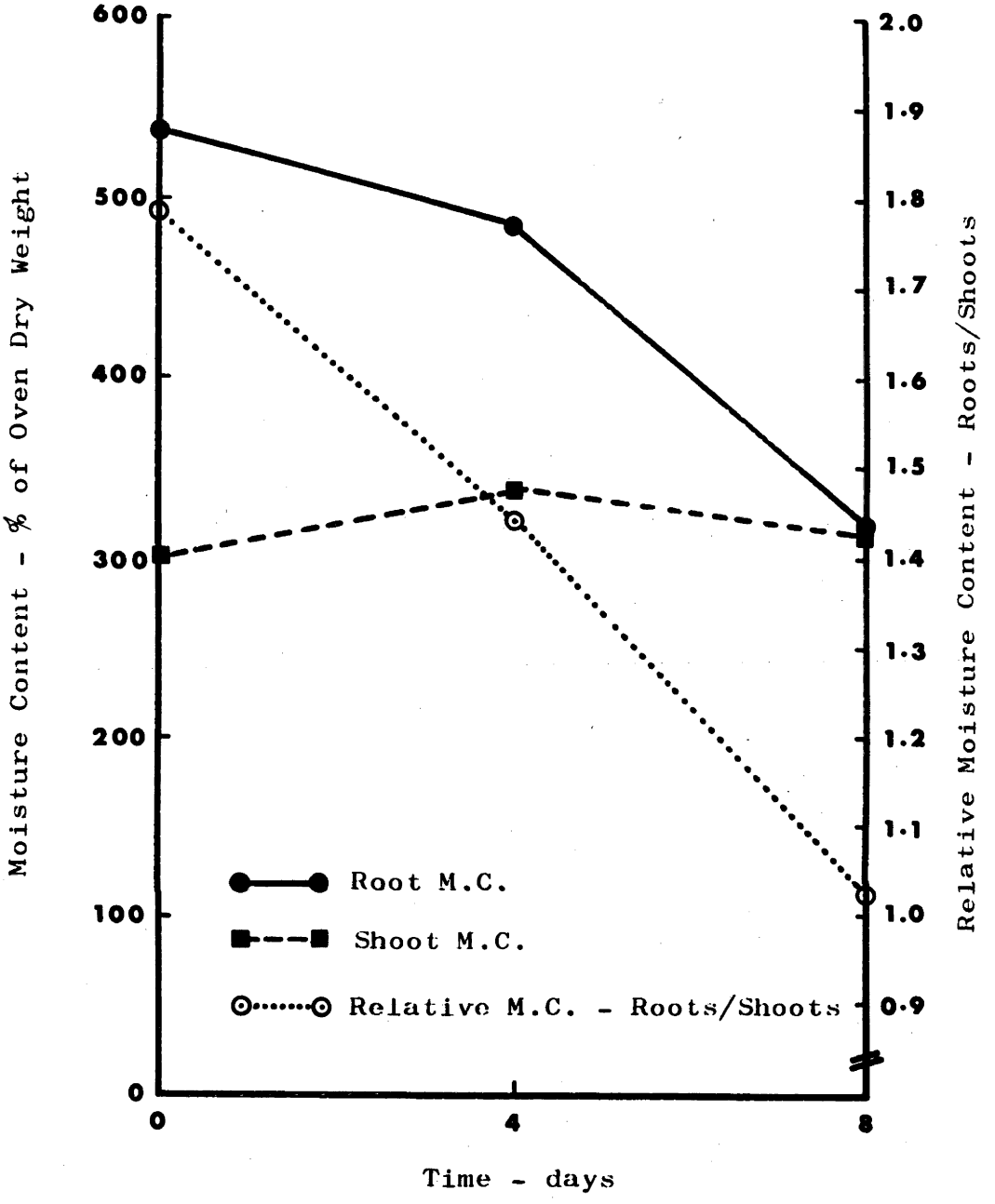


FIGURE 8 : Variation in the moisture content of roots and shoots and in the relative moisture content of roots/shoots during the onset of drought in 5-month-old P. radiata seedlings. Each point represents the average of three replicates.

increasing soil moisture stress on the relative moisture content of roots/shoots is to depress the ratio from 1.8 under well watered conditions to 1.0 under severe stress conditions. These ratios were used as the basis for estimating the actual soil moisture content of pots (see Appendix 7 for method) assuming that the relative water contents of roots/shoots in Treatments A, B, C, D, and E were 1.0, 1.2, 1.4, 1.6, and 1.8 respectively.

Height growth was assessed at monthly intervals from the time treatments were first applied on 12/10/65 (seedlings 3 months old) until the time of termination of the experiment 10 months later. At this stage, one seedling from each treatment was selected for the measurement of CO_2-exchange. After removing its terminal shoot, the net photosynthesis of fully expanded 7- to 8-month-old foliage was measured at 25 $\pm$ 1°C and 2500 $\pm$ 100 ft.c. The gas analysis was conducted at both extremities of the watering regime to which each plant was being subjected at the time, and the relative water content of foliage was recorded simultaneously. Meanwhile, the other two seedlings in each treatment were harvested and their root, shoot, and whole plant oven dry weights were determined.

While only one seedling from each treatment was submitted to gas analysis, it was hoped to obtain several replications with time. It was found in each treatment, however, that the photosynthetic capacity of the seedling, though at the same soil moisture content but in the next drying cycle, increased markedly with time (Figure 9). Thus, replication in time was not

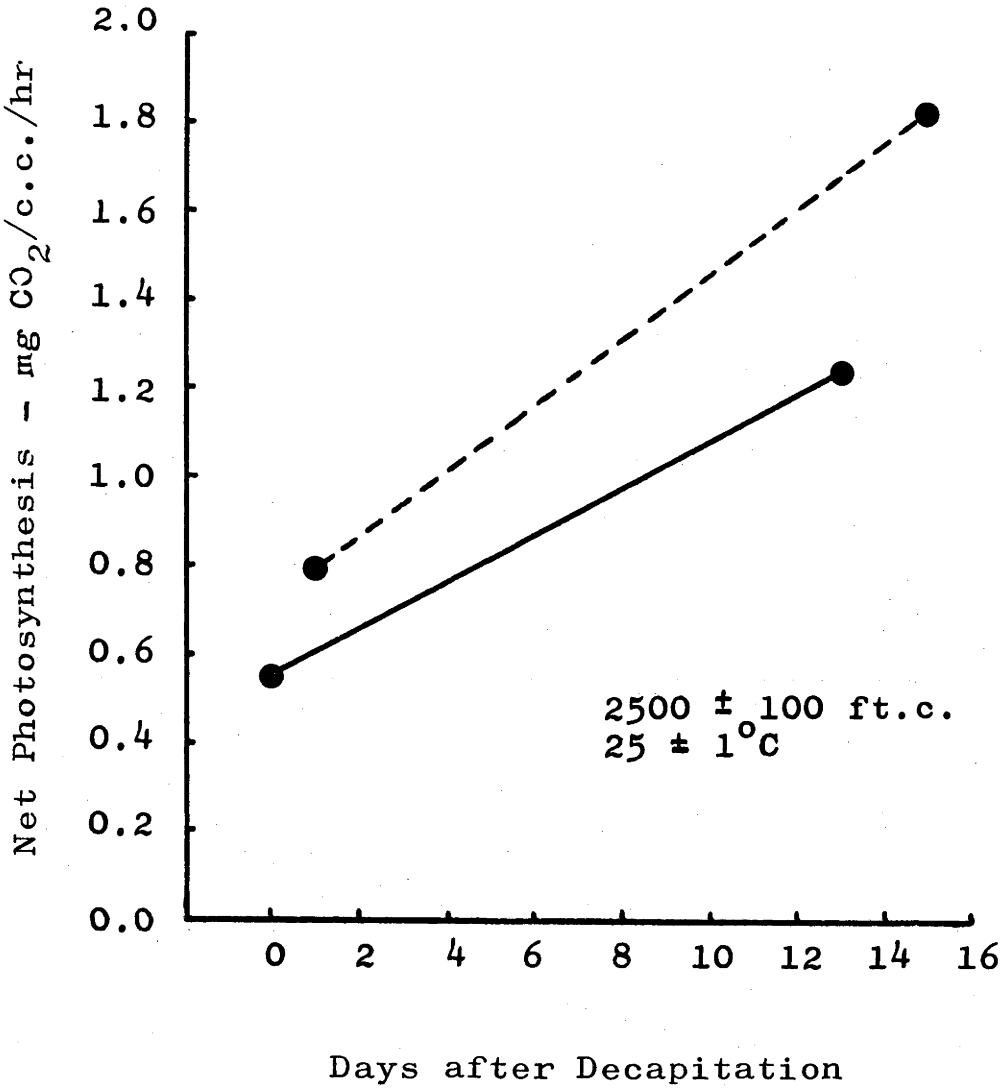


FIGURE 9: Variation with time in the rate of net photosynthesis of a P. radiata seedling following decapitation (seedling drawn from Treatment E of Experiment 3, Study 3).

●——● Lower end of watering range

●- - -● Upper end of watering range

possible, and the results presented in Figure 17 are of necessity based on one determination of net P at either end of the range of each watering regime. As these determinations were made, in all cases, less than four days after severance of the terminal shoot, the data presented are valid (it is demonstrated in a later study that severance of the terminal shoot has little effect on the CO_2-exchange capacity of the remaining foliage in the first four days following severance).

The cause of the unexpectedly marked increase in the photosynthetic capacity of foliage with time, referred to above, was the subject of subsequent experimentation which is described later.

Note: Limitations imposed by the gas analysis system and time prevented replication of treatments in Experiments 1 and 2 and of the gas analysis measurements in Experiment 3. The trends established in these trials are thus tentative.

RESULTS

The main features of the results of the several experiments are summarised in Table 8. Supporting data are presented in Tables 9-10, Figures 10-17, and Plate 5.

DISCUSSION

Experiment 1 - Drought experiment

The rapid decline in the rate of net P and transpiration, when the RWC of foliage fell below approximately 82 per cent, occurred at a low soil

TABLE 8: Summary of the main features of interest in the results presented demonstrating the short and long term responses of *P. radiata* seedlings to conditions of soil moisture deficit and excess respectively

Experiment	Features of interest	Reference
1 Soil moisture deficit	1. Rapid fall in the rate of net P once the RWC fell below approximately 82 per cent	Figures 10 & 11
	2. Effect of severity of the drought cycle on the rate of recovery of net P following rewatering	Figure 11
	3. Occurrence of maximum rate of net P in the 3rd and 4th cycles at comparatively low soil moisture levels	Figure 11
	4. The lesser effect of soil moisture stress on dark respiration than on CO_2-uptake	Figures 10 & 11
	5. Sudden burst of respiration just prior to seedling death	Figure 10
	6. Lethal foliage RWC lay between 40-50 per cent	Figures 10 & 11
2 Soil moisture excess	7. Rapid fall in rates of net P and transpiration soon after the onset of flooding	Figure 12
	8. Diurnal pattern of CO_2- and H_2O-vapour exchange in the flooded seedling was an early morning peak followed by a rapid fall to a stable level	Figure 12
	9. Ability of plant to maintain net P and transpiration at a reasonable level (average approximately 60 per cent of initial) for up to five weeks under flood treatment	Figure 13
	10. Marked upsurge in transpiration on Day 29	Figure 13
	11. High RWC (92.8 per cent) on Day 42	Figure 13
3 Range of soil moisture regimes	12. Pattern of height increment and absolute height growth. Treatments D and A best and poorest respectively throughout. Treatment E variable	Tables 9 & 10 Figures 14 & 15
	13. Dry matter production reduced in Treatment A. Production of B, C, D, and E comparable.	Figure 16
	14. Low rates of net P of foliage in Treatment A.	Figure 17

TABLE 9: Effect of various watering regimes on the mean height (cm) of P. radiata seedlings from age 3-13 months. See text for details of the watering regimes applied. Each entry represents the average of three seedlings.

Age (months)	Date	Watering regime (treatment) A	B	C	D	E	Standard error	Significance of treatment effect ϕ	L.S.D. (p<.05)
3	12.10.65	11.4	11.5	11.4	11.3	11.6	±0.1	-	-
4	12.11.65	23.0	22.1	23.7	24.9	26.1	±0.6	*	2.4
5	12.12.65	38.3	39.4	42.6	45.6	45.5	±1.1	**	4.3
6	12. 1.66	49.2	57.4	60.5	64.8	60.4	±1.1	***	4.2
7	12. 2.66	52.8	66.3	68.1	73.9	66.3	±1.5	***	5.9
8	12. 3.66	55.5	73.4	74.0	81.0	70.6	±2.0	***	8.2
9	12. 4.66	59.4	77.8	80.3	85.9	73.7	±2.2	***	8.6
10	12. 5.66	63.1	82.2	85.9	90.5	76.8	±2.3	***	9.4
11	12. 6.66	64.2	85.4	90.4	95.6	83.0	±2.3	***	9.3
12	12. 7.66	64.8	88.3	94.2	100.5	89.4	±2.4	***	9.4
13	12. 8.66	66.1	92.9	99.9	108.6	97.2	±2.1	***	8.5

TABLE 10: Effect of various watering regimes on the mean monthly height increment (cm) of P. radiata seedlings between the ages of 3-13 months. See text for details of the watering regimes applied. Each entry represents the average increment of three seedlings.

Age (months)	Calendar months	Watering regime (treatment) A	B	C	D	E	Standard error	Significance of treatment effect ϕ	L.S.D. (p<.05)
3-4	Oct/Nov	11.6	10.5	12.3	13.6	14.5	±0.6	**	2.3
4-5	Nov/Dec	15.4	17.3	18.9	20.7	19.4	±0.7	**	2.9
5-6	Dec/Jan	10.9	18.0	17.9	19.2	14.9	±0.7	***	2.6
6-7	Jan/Feb	3.6	8.8	7.6	9.0	5.9	±0.8	**	3.2
7-8	Feb/Mar	2.7	7.2	5.9	7.1	4.3	±0.7	*	3.0
8-9	Mar/April	3.8	4.4	6.3	4.9	3.1	±0.6	*	2.4
9-10	Apr/May	3.8	4.4	5.7	4.6	3.0	±0.6	NS	-
10-11	May/June	1.0	3.2	4.5	5.1	6.3	±0.6	**	2.2
11-12	June/July	0.6	2.9	3.8	4.9	6.4	±0.6	**	2.4
12-13	July/Aug	1.3	4.6	5.6	8.2	7.8	±0.6	***	2.4

ϕ NS Non-significant
* Significant at p<.05
** Significant at p<.01
*** Significant at p<.001

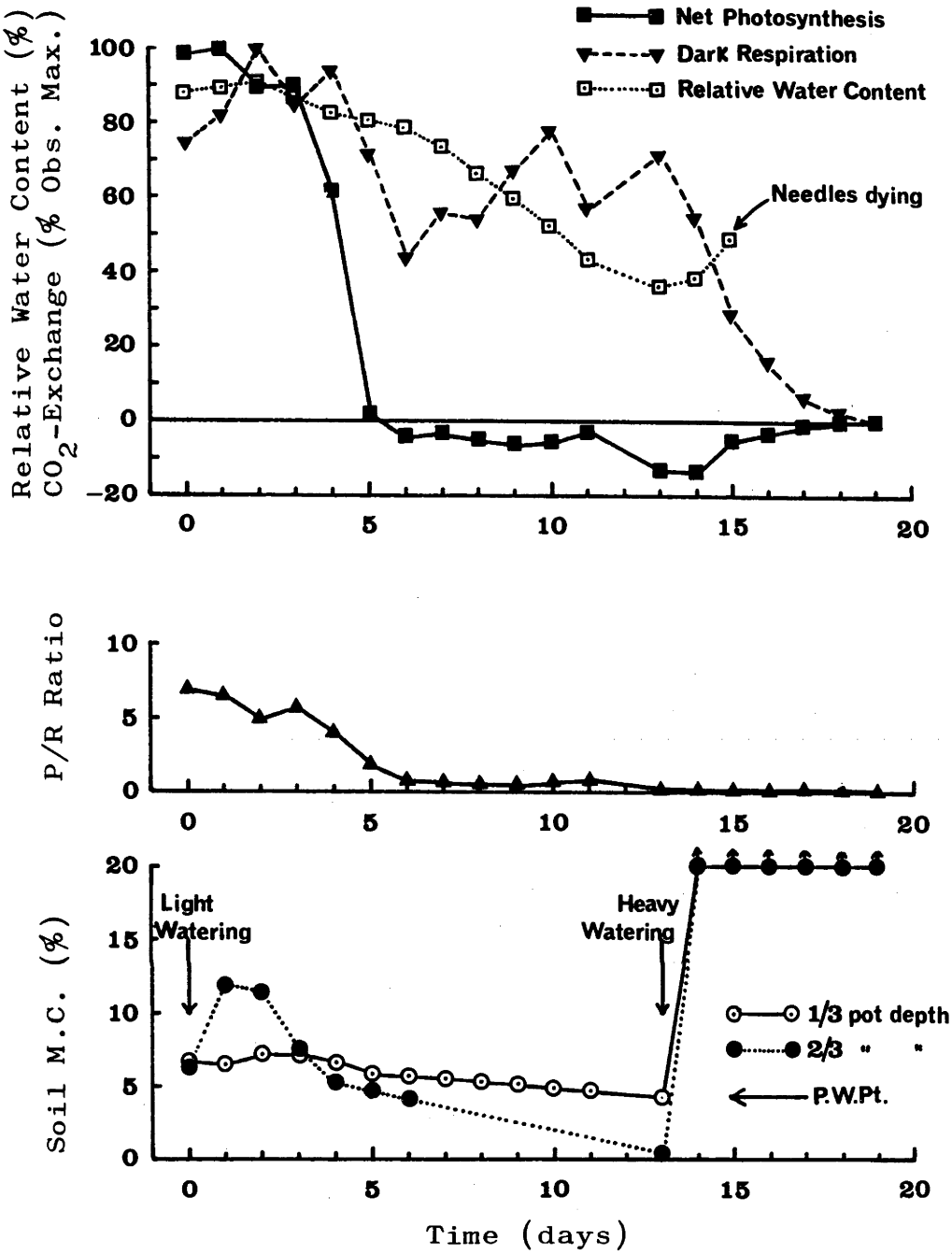


FIGURE 10: Effect of variation in soil moisture on CO_2-exchange and the relative water content of foliage on a potted 2-year-old P. radiata seedling growing in a controlled environment - Illumination 2500 ± 100 ft.c.; photoperiod 12 hr; day temperature 25 ± 1°C; night temperature 20 ± 1°C.

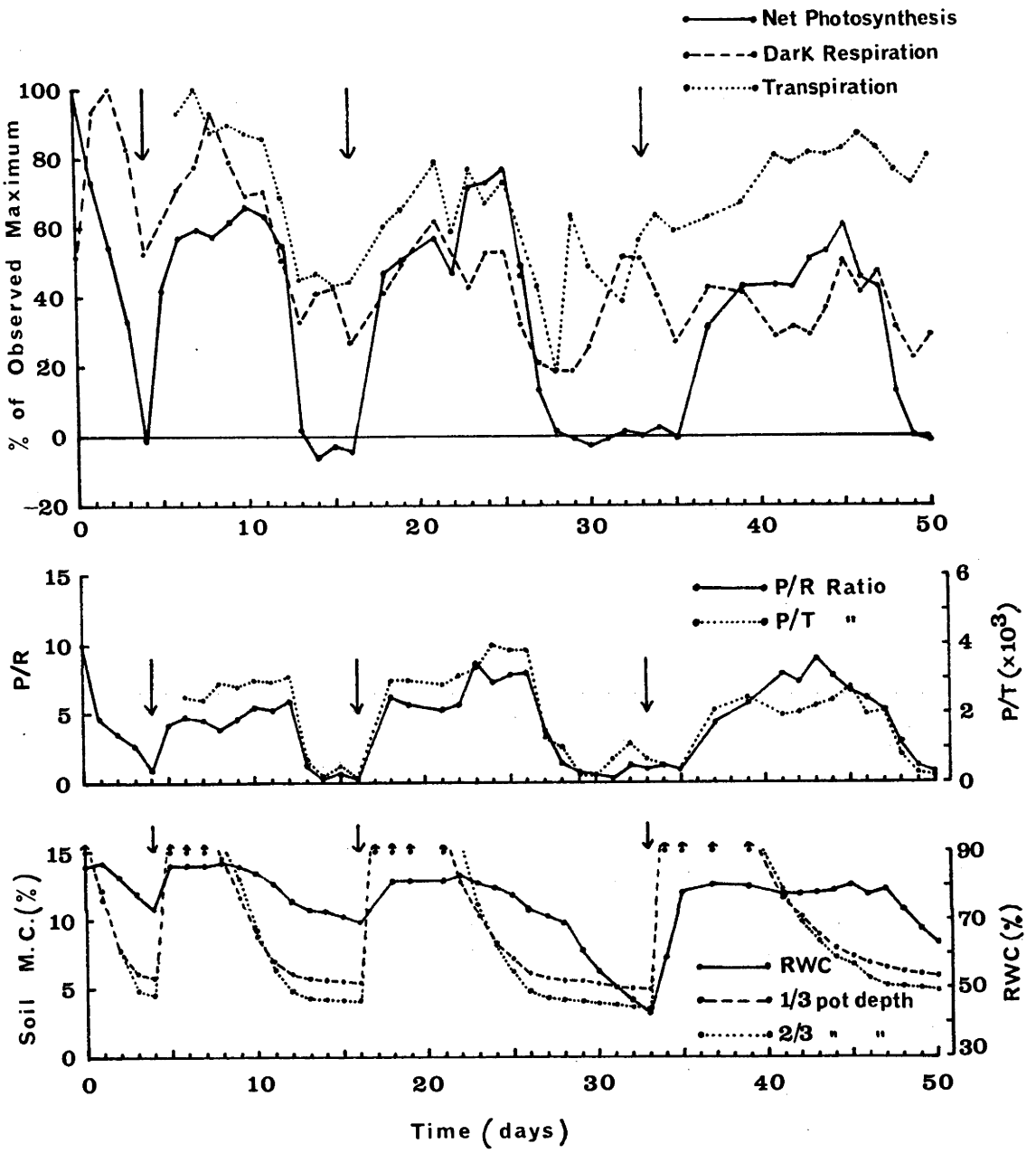


FIGURE 11: Effect of variation in soil moisture on CO_2- and H_2O— vapour exchange and on the relative water content of foliage on a potted 2-year-old P. radiata seedling growing in a controlled environment - Illumination 2500 ± 100 ft.c.; photoperiod 12 hr; day temperature 25 ± 1°C; night temperature 20 ± 1°C. Arrows indicate time of re-watering (↓).

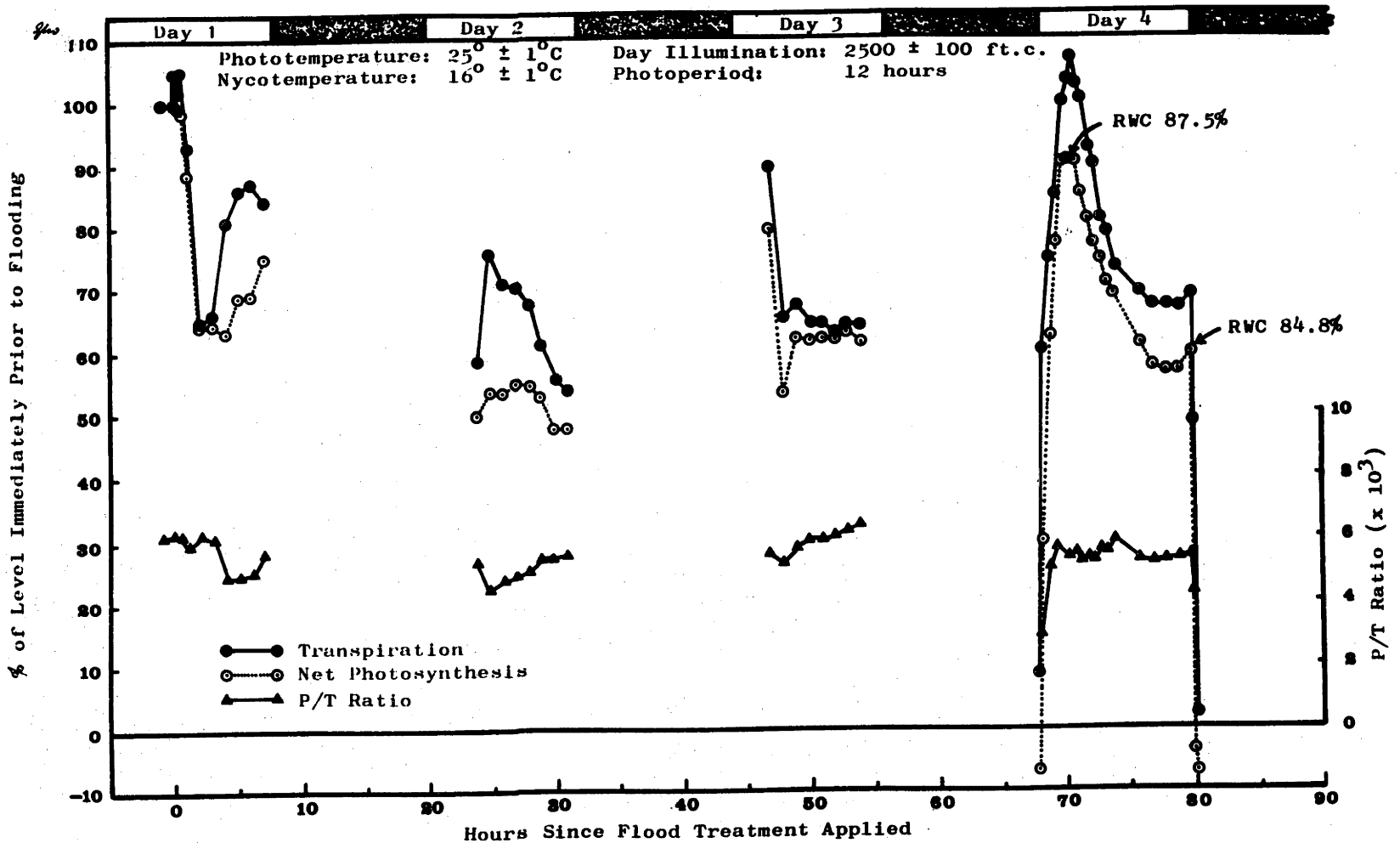


FIGURE 12: Short term changes in net photosynthesis, transpiration, and in the P/T ratio on flooding the soil of a potted P. radiata seedling.

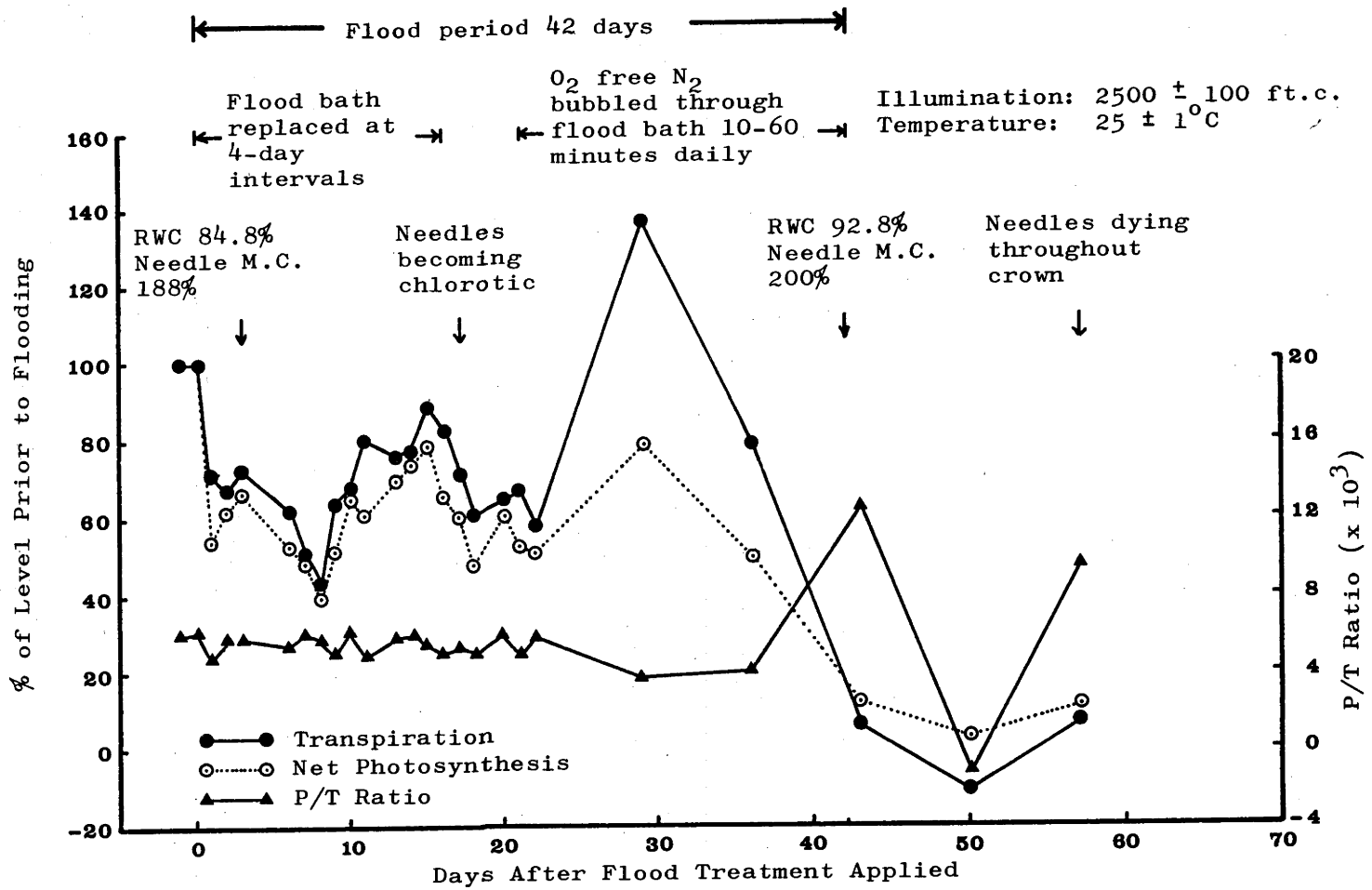


FIGURE 13: Long term changes in net photosynthesis, transpiration, and in the P/T ratio on flooding the soil of a potted P. radiata seedling.

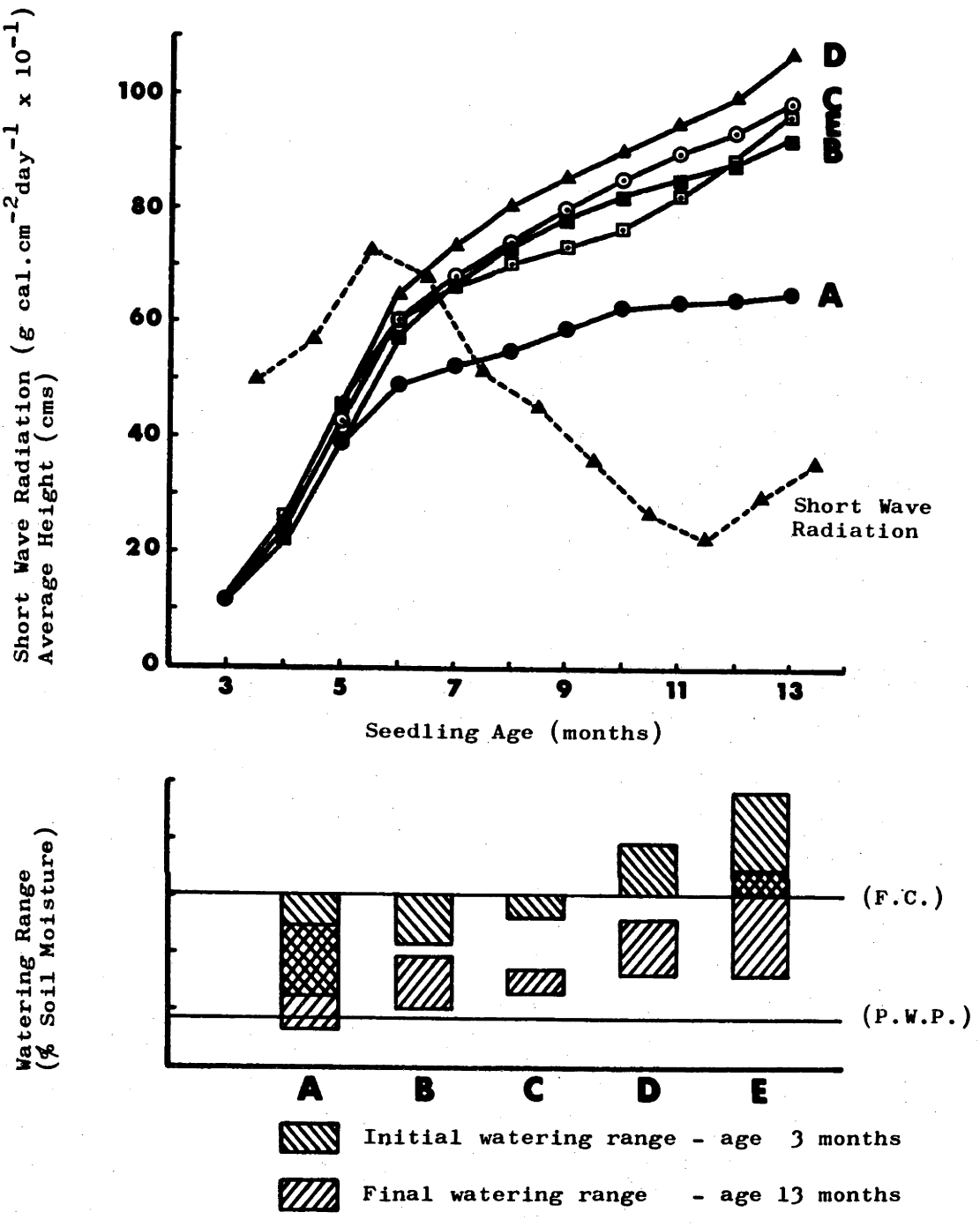


FIGURE 14 : Effect of various watering regimes on the absolute height growth of P. radiata seedlings. Treatments applied as from three months of age. Each entry is an average of three replicates.

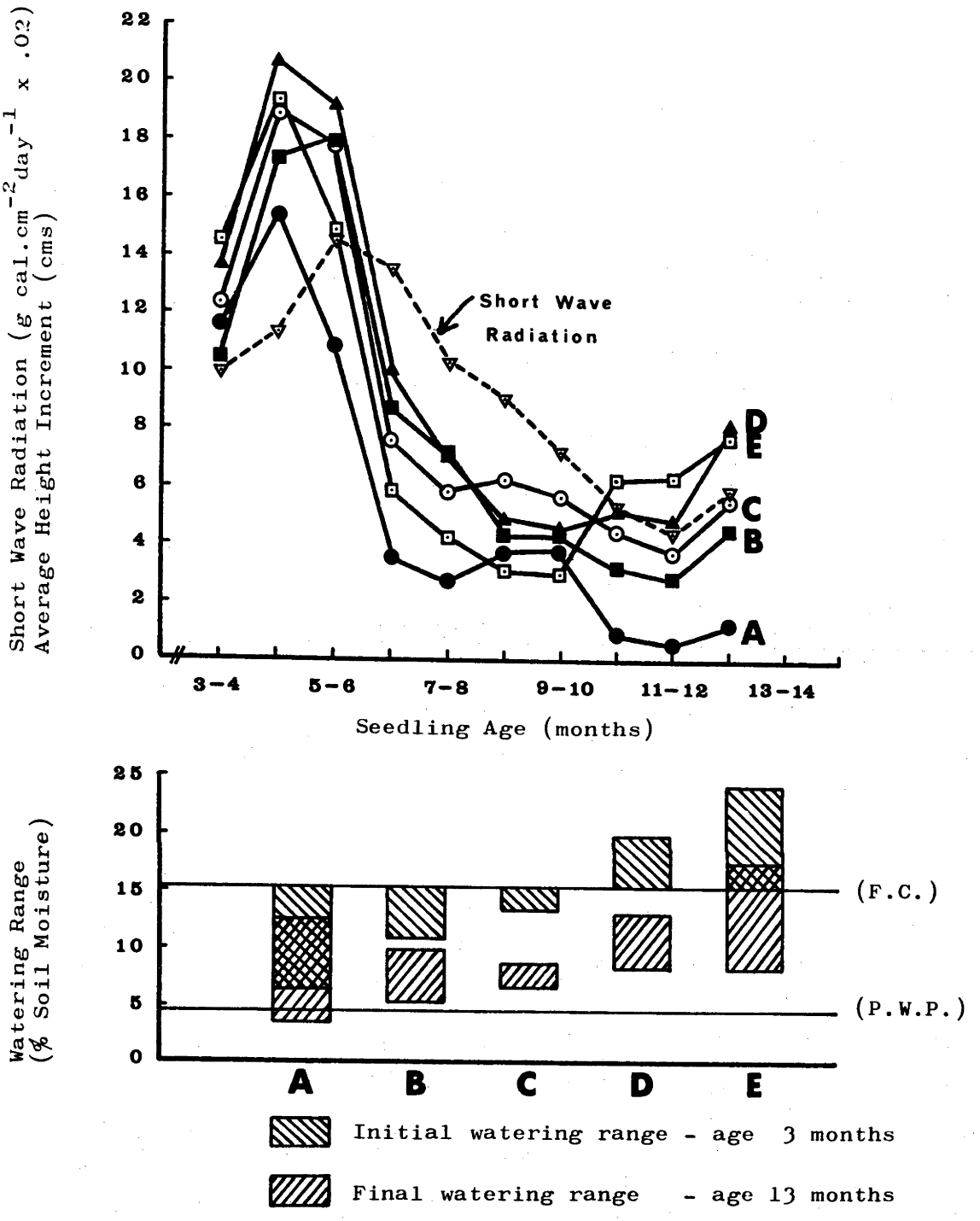


FIGURE 15: Effect of various watering regimes on the monthly height increment of P. radiata seedlings. Treatments applied as from three months of age. Each entry is an average of three replicates.

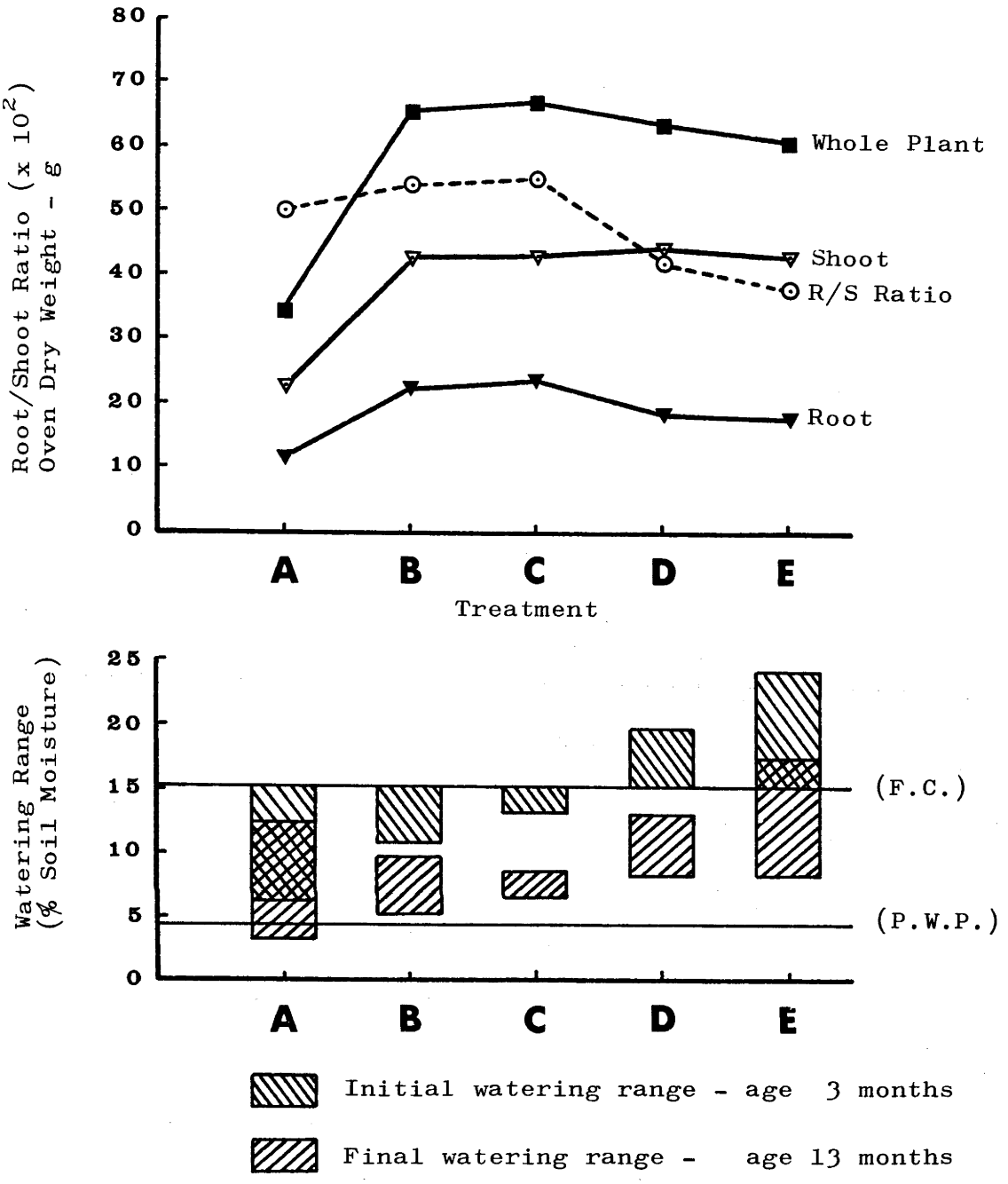


FIGURE 16 : Effect of various watering regimes on the dry matter production of roots, shoots, and whole plants, and on the root/shoot ratio of Pinus radiata seedlings. Treatments applied as from three months of age. Each entry is an average of two replicates.

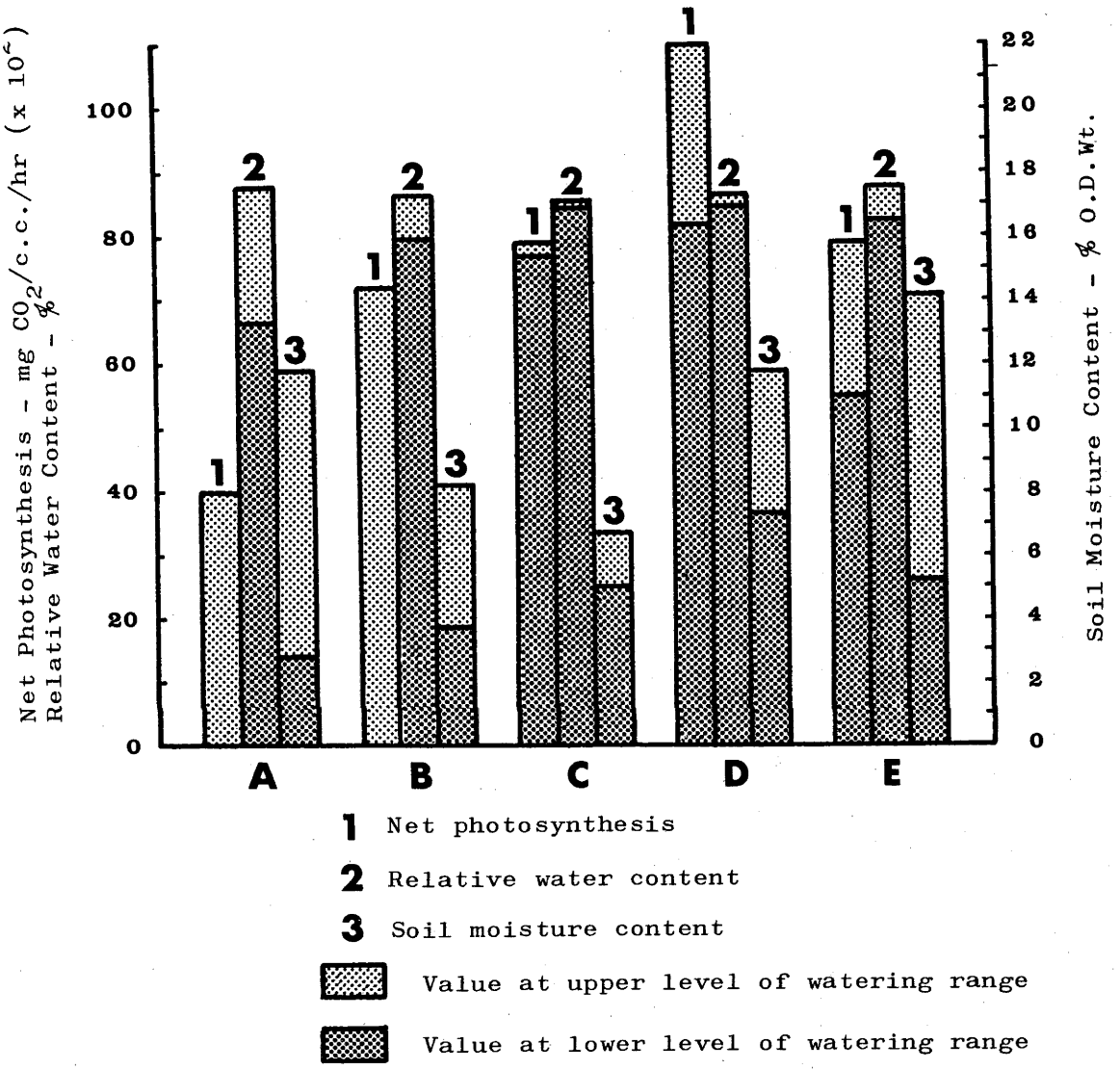


FIGURE 17: Net photosynthesis of Pinus radiata seedlings under each of five watering regimes as related to the soil moisture content and the relative water content of foliage. Illumination 2500 ± 100 ft.c. Temperature 25 ± 1°C. Measurements based on one seedling only per treatment.

PLATE 5 The development of seedlings in Experiment 3 (Study 3) at age 13 months after 10 months of treatment under various watering regimes, viz.,

A. Droughted

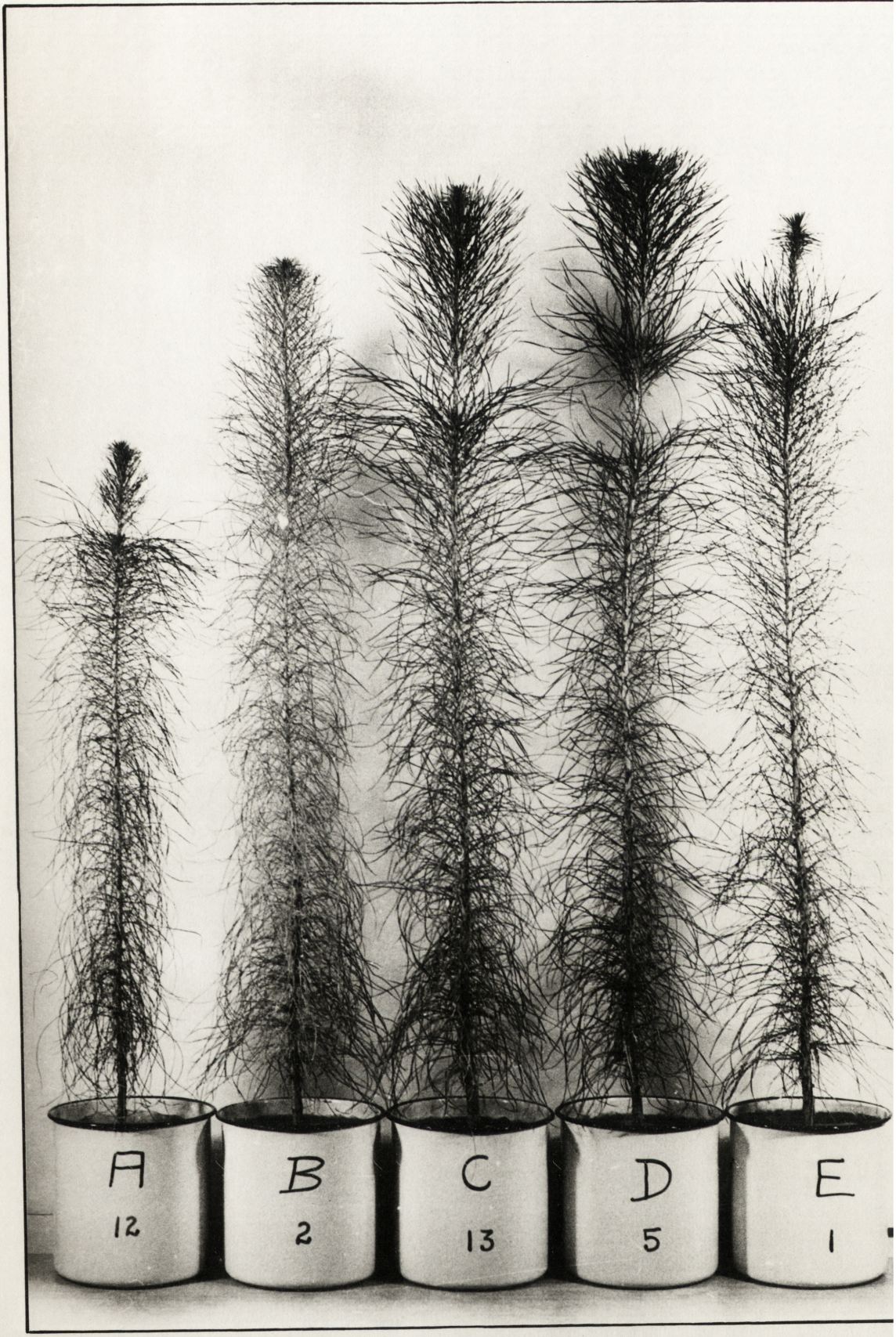
B. Semi-droughted

C. Moderate watering

D. Well watered

E. Heavily watered

Scale: Height of 'C' ≈ 1 metre.


A
12
B
2
C
13
D
5
E
1

moisture content, viz., 5-7 per cent (Figures 10 and 11). This suggests that over the greater part of the range of soil moisture availability in the particular soil used, seedling physiological activity was largely unaffected by variation in soil moisture content. The probable explanation is that the structure of the potting soil was sufficiently coarse to allow water to be freely absorbed by the plant roots over the greater part of the available range. This is supported by the data presented in Table 7 which show little variation in the RWC of foliage at any insertion level on the stem, as the soil water content fell from 15 per cent to 7 per cent (FC $\approx$ 15.2 per cent, PWP $\approx$ 4.4 per cent).

The rate of recovery of net P on rewatering following drought is obviously determined by the severity of the drought cycle (Figure 11; and cf. Evdokimova, 1964). Following the severe third cycle, net P remained virtually zero until the third day following rewatering. Recovery periods of 2-3 days in the gas exchange and metabolic processes of plants following drought are commonly observed (Jarvis, 1963; Ben-Zioni et al., 1967) and periods of up to two weeks are not unknown (Brix, 1962).

The observation that maximum CO_2-uptake in each drought cycle occurs at a soil moisture content in the lower half of the available range (Figure 11) is not unexpected. Assuming that the moisture characteristic of the potting soil is such that water is freely available to the plant roots over the greater part of the available range, then, following rewatering after a drought cycle, the recovery pattern of the

photosynthetic mechanism would continue until the 'limiting' soil moisture level was again reached.

The less marked effect of a plant water stress on dark respiration than on net P (Figures 10 and 11) may be attributed to stomatal closure having little influence on respiration, the concentration of O_2 in the air being so high that it seldom limits the respiratory process even when the stomates are closed (Zeigler - seen in Huber, 1964). The burst of CO_2-evolution between Days 9-13 (Figure 10) occurred at the stage when the soil moisture content was very low ($\approx$1.4 per cent - determined gravimetrically) and the plant had a severely desiccated appearance. It is thought to represent the climacteric increase in CO_2-production which often occurs just before the metabolic disintegration of plant tissue.

The needle RWCs (corrected) from which seedling B recovered in the 1st, 2nd, and 3rd drying cycles (Figure 11) were approximately 76, 72, and 50 per cent respectively. As seedling A had previously succumbed to a RWC (corrected) of approximately 39 per cent (Figure 10), the lethal RWC for the needles of the seedlings sampled probably lay between 40 and 50 per cent (cf. Johnston, 1959; Oppenheimer, 1968) - the lethal RWC for many tree species has been found to fall within this range, e.g., see Jarvis (1963), Jarvis and Jarvis (1963), Pharis (1966), Pook et al. (1966). The partial recovery of leaf turgor between Days 13-16 (Figure 10), though the plant subsequently died, suggests that death of the root occurred while the stem and needles were still alive and able to take up

water. Similar findings have been reported by Brix (1960), Ursic (1961), and Pharis (1966).

Experiment 2 - Flood experiment

The physiological responses observed in this study were not complicated by growth of the seedling, as repeated measurement of seedling height, and of the length of needles at various positions on the stem, indicated that extension growth ceased when flood treatment was applied.

The rapid fall in net P and transpiration in the first two hours after flooding (Figure 12) is attributed to a plant water stress induced by a depressed water absorption by the roots. Indirect evidence that this occurred was obtained by flooding another seedling for two hours and then severing its shoot from its root system under water. The response of net P and transpiration, which was followed for 90 minutes after severance, is illustrated in Figure 18. The rapidity with which both gas exchange processes were stimulated ($\approx$ 160 per cent of initial within 30 minutes) was far in excess of that found in normally watered plants on severance under water ($\approx$ 110 per cent - see Figure 23). It is significant that the stimulation evident in Figure 18 is of the same order as the depression of net P and transpiration evident in Figure 12, which suggests that severance under water (of the shoot from the root system) released a shoot water deficit which existed in the intact flooded plant. It has been demonstrated by others that the O_2-concentration of a soil is rapidly depleted by flooding (Papendick and

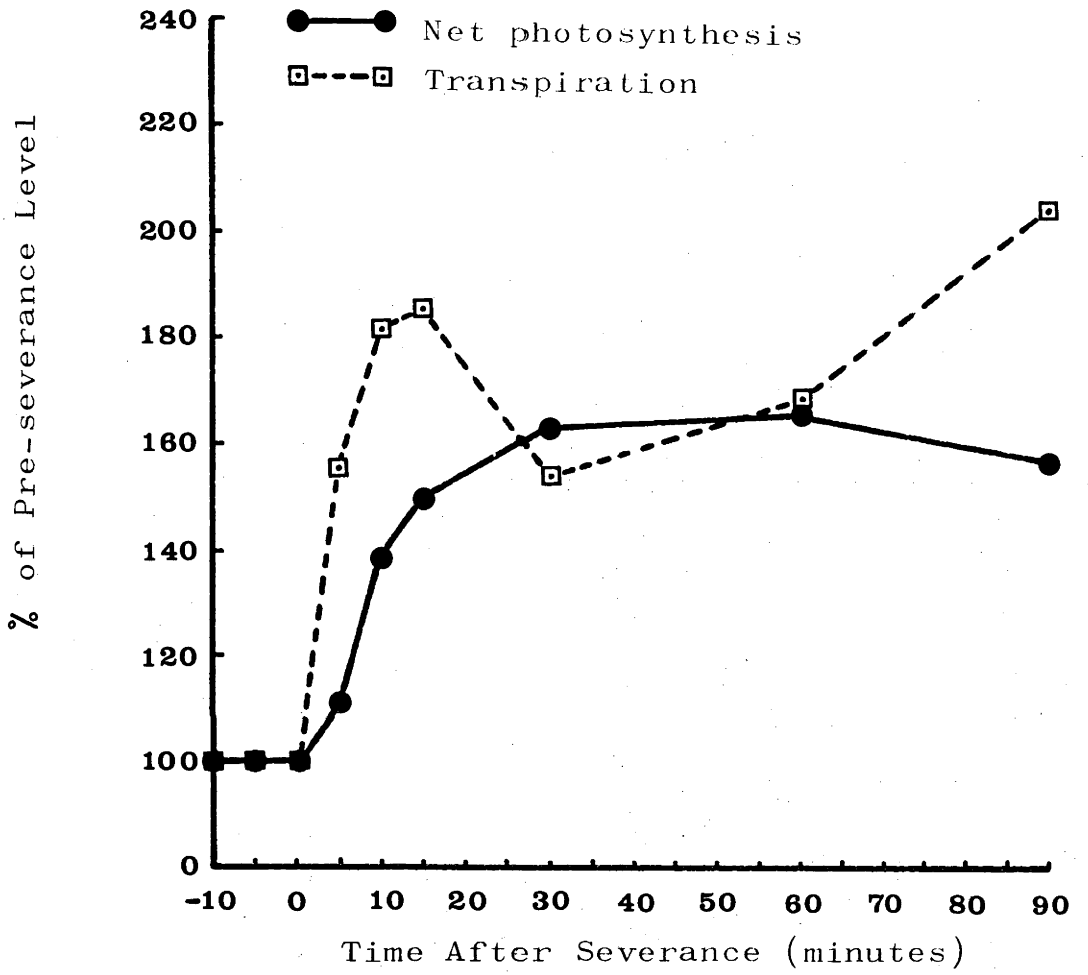


FIGURE **18**: Response of net photosynthesis and transpiration in the shoot of a flooded P. radiata seedling following severance from the root system.

Runkles, 1966; Ponnamperuma et al., 1967) and that the development of anaerobic conditions in a soil leads to a marked reduction in the rate of passive water absorption (Kramer, 1951; Pearson, 1966; Currier, 1967).

The pattern of plant response on Day 3 (Figure 12) suggests an early morning peak in both net P and transpiration. The pattern is clarified on Day 4 when the response was monitored continuously from 0600 hours to 1830 hours. The attainment of the early morning maximum in both exchange processes is probably due to the plant having eliminated its water deficit overnight. Throughout the experimental period, the rates of photosynthesis and transpiration were essentially in phase (Figures 12 and 13); this implies that they were both being regulated by the same controller. The only feasible controller is stomatal aperture.

The fact that both net P and transpiration varied around 60 per cent of the pre-flood levels of exchange for up to five weeks after treatment, leads one to conclude that O_2 was reaching the root system. If it did not, the flow of respiratory energy would cease and membrane structure and function would collapse (Slatyer, 1967). It is unlikely that convection currents in the flood bath would have carried sufficient of the gas to the plant roots from the growth cabinet atmosphere since diffusion of O_2 into a flooded soil occurs at a negligibly slow rate (Clark and Kemper, 1967). Recent work indicates that in some species of plants, the O_2 requirement of a flooded root system may be met by downward diffusion through the stem (Kursanov, 1966;

Greenwood, 1967a, b; Letey and Stolzy, 1967). It is not known if the phenomenon occurred in this experiment.

The marked upsurge in the rate of transpiration on Day 29 (Figure 13) is probably evidence of the widespread death of roots and of an associated temporary reduction in resistance to water movement across the root cortex and, hence, increase in water absorption (Kramer, 1951; Oppenheimer, 1968). This supposition is supported by the fact that in the ensuing three weeks, there was a regular fall in transpiration (and net P) to a negligible rate by Day 50. When the flood conditions were removed on the 42nd day, the root ball was turned out of the pot for examination. No living roots were found on its surface and the stench was typical of rotting plant matter as is found in a bog. Despite the fact that the shoot of the plant exhibited severe chlorosis, the distal ends of the needles were dead (approximately 10 per cent of the total length), and the needles themselves were stiff and desiccated in appearance, a measurement of the water status of foliage revealed an unexpectedly high relative water content of 92.8 per cent. A satisfactory explanation cannot be offered for this high RWC value though current work suggests that high RWCs may be general in seedlings flooded for some time (Bacon and Wood, unpublished data). It was thought that the metabolic activity of the plant may have been so disorganised by the flood treatment that the foliage had lost its ability to regain turgor when placed in a saturated atmosphere. However, the moisture content of the foliage was found to be 200 per cent of the oven

dry weight. A moisture content of this order was typical of normal healthy seedlings in the batch of stock from which the experimental plant was drawn.

Following the removal of the flood bath, the experimental plant shed all its foliage but the terminal bud remained alive. The plant remained 'dormant' for the next two months, but then the terminal bud and basal axillary buds began to expand. An estimate of the survival period for the seedling under the flood conditions imposed is thus 6-7 weeks.

Experiment 3 - Height growth and dry matter production under various soil moisture regimes

A significant effect of soil moisture regime on height increment was obvious within one month of treatment (Table 10). The good initial growth in the wettest treatment (E) is explained if it is assumed that the root systems of the young seedlings at that stage were concentrated in the upper levels of the pot. The watering regime of Treatment E did not flood the pot, but it would have saturated an unknown depth of soil at the bottom. The soil above this saturated section would have been at field capacity and in an optimum condition for plant growth. The significant fall in the height increment of Treatment E in the third month of treatment (Table 10) is interpreted as evidence of the extension of root systems into the lower levels of the pot where they would have been subjected periodically to flooding. As was demonstrated earlier, anaerobic conditions markedly affect plant response. The subsequent recovery of height increment in Treatment E (age 10-13 months) is attributed to a

lessening of the severity of the watering treatment due to the growth of the plant. As previously mentioned, treatments were applied by watering to pre-determined weights, and corrections for plant weight were not made during the course of the experiment. At the end of the experiment, Treatment E, because of the increased weight of the plant, actually involved a watering regime which gave a soil moisture content range of 8-18 per cent. This would have been ideal for plant growth.

Treatment A obviously subjected the plants to stress from the outset and resulted in consistently low height increments. Reduced cell enlargement may be associated with these low increments (Slatyer, 1967).

The general low level of monthly height increments from January to August (Table 10) is due to seasonal conditions. The seasonal fall in increment is responsible for the falling level of significance in the treatment effect from January to May.

The average moisture contents of shoots in Treatments A, B, C, D, and E at the harvesting of the experiment were 170, 184, 199, 211, and 216 per cent respectively. As shoot dry matter production in Treatments B, C, D, and E was comparable (Figure 16), it is apparent that unfavourable soil moisture conditions affect fresh weight more than dry weight yield (cf. Bierhuizen, 1961). One may also conclude that elongation of the shoot is possibly more sensitive to moisture stress than is the production of dry matter (cf. Hagan and Vaadia, 1960) as there are significant

differences in height between some of the treatments which produced the same amount of dry matter (Table 9, Figure 16). This conclusion is tenuous, however, as it is not uncommon in pot experiments to find a restriction of root volume eventually eliminating differences between treatments in dry matter production (Hopkinson, 1966).

The observation that the rate of net P of foliage in Treatments A and B is negligible at relative water contents less than 80 per cent (Figure 17), is in accord with earlier findings that the net P of droughted seedlings fell rapidly to zero once the RWC fell to 80-82 per cent. Possibly the threshold value of the RWC at which stomatal closure occurred in the potted P. radiata stock used in this project lay between 80-82 per cent.

STUDY 4

EFFECT OF DECAPITATION OF A SEEDLING ON THE GAS EXCHANGE CAPACITY OF ITS NEEDLES

It was observed in the previous study that the rate of photosynthesis of needles on P. radiata seedlings increased greatly with time following removal of the shoot apex (Figure 9). One aim of this study was to confirm the effect observed, to determine whether it is due to the removal of the apical bud and/or developing foliage, and to establish the site of the effect in the photosynthetic system. The other aim was to investigate the possibility of developing a technique, for later application in the field, of severing a shoot from a P. radiata seedling without seriously interfering with the pattern of CO_2-exchange of the shoot. The first aim was realised in Experiments 1 and 2, and the second in Experiment 3.

MATERIALS AND METHODS

Experiment 1

Three potted 8-month-old P. radiata seedlings of uniform height and development were subjected to gas analysis as follows:

Treatment A - Control treatment. The intact upper 20 cm of the terminal shoot were enclosed in the assimilation chamber.

Treatment B - The upper 12 cm section of the terminal shoot was decapitated and the 8 cm section below the point of severance was enclosed in the assimilation chamber.

Treatment C - The terminal 20 cm section of the shoot was enclosed in the assimilation chamber after carefully stripping the foliage from the upper 12 cm section, but leaving the apical bud and the terminal rosette of developing primary leaves intact.

Prior to gas analysis, the stump of the seedling in Treatment B and the stripped section of the stem in Treatment C were smeared with vaseline and covered with aluminium foil to prevent water loss from the severed and defoliated parts. Net P was measured at an illumination of 2500 $\pm$ 100 ft.c. and a temperature of 25 $\pm$ 1^{o}C on each of four occasions, viz., immediately after preparing the shoot, and on the 4th, 8th, and 15th days after preparation. The analysis was conducted on Treatments A, B, and C at 1200, 1400, and 1600 hours respectively. The rates of CO_2-exchange are expressed per unit of foliage volume which was measured at the completion of the experiment.

Experiment 2

Two potted 15-month-old P. radiata seedlings were prepared for gas analysis by severing the shoot of one seedling at the 85 per cent level of stem height (Treatment D) and that of the other at the 66 per cent level of stem height (Treatment E). The needles just below these points of severance were judged to be representative of the youngest fully expanded foliage

and mature foliage on the stems respectively. The cut stubs were smeared with vaseline and covered with aluminium foil as previously.

Light response curves for CO_2- and H_2O-vapour exchange were defined by measuring the exchange rates in a descending series at 3000, 2000, 1000, 500, and 0 ft.c. at 25 $\pm$ 1^{o}C on the upper 10 fascicles of each de-topped plant. Measurements were made immediately after the initial severance of the top, and on four occasions thereafter at intervals of 3-6 days. The lengths of all needles used in the gas exchange measurements were recorded immediately after the completion of each run (Table 11) and the volume of foliage was recorded on completion of the experiment. The volume at earlier dates was then estimated, using the percentage increase in growth data obtained from the measurements of needle length. The measurement of exchange rates was discontinued on the 17th day after severance as there was evidence that axillary buds were beginning to expand.

TABLE 11 Mean length (cm) of needles used in gas analysis during the 17-day period after shoot decapitation

Days after severance	Treatment D (severed at 85 % level)	Treatment E (severed at 66% level)
0	14.7	16.8
3	15.1	16.8
7	15.5	16.8
11	15.9	16.9
17	16.1	16.9

Experiment 3

The stock comprised 16-month-old potted P. radiata seedlings, raised under glasshouse conditions from controlled cross seed, and acclimatised for one week in a controlled environment chamber under the test conditions of light and temperature, viz., 3100 ± 100 ft.c. and 25 ± 1°C respectively.

A number of preliminary experiments indicated that the technique of severing the shoot initially in air and immediately recutting under water was successful occasionally, but the response more often was unpredictable (cf. Cameron, 1964). The results suggested that this was due either to air embolism effects or to the blockage of tracheids with resin. The technique which was finally adopted involved making the initial cut under water. The procedure was as follows (see Figure 19):

1. A rigid plastic container (B), open at the top and with a slit extending vertically down one side to a circular hole (C) in the bottom, was fitted to the experimental plant so that it enclosed a portion of the stem. A sleeve of polythene film (A) was fitted over the shoot of the plant and moved down the stem until it enclosed the container (B). The lower end of the polythene sleeve was then tied securely around the stem so that the junction (D) was watertight. After tucking the upper end into the cavity of the plastic container, the sleeve was made secure by tying with tape at each of two positions (E). The stem of the plant was then clamped at F.

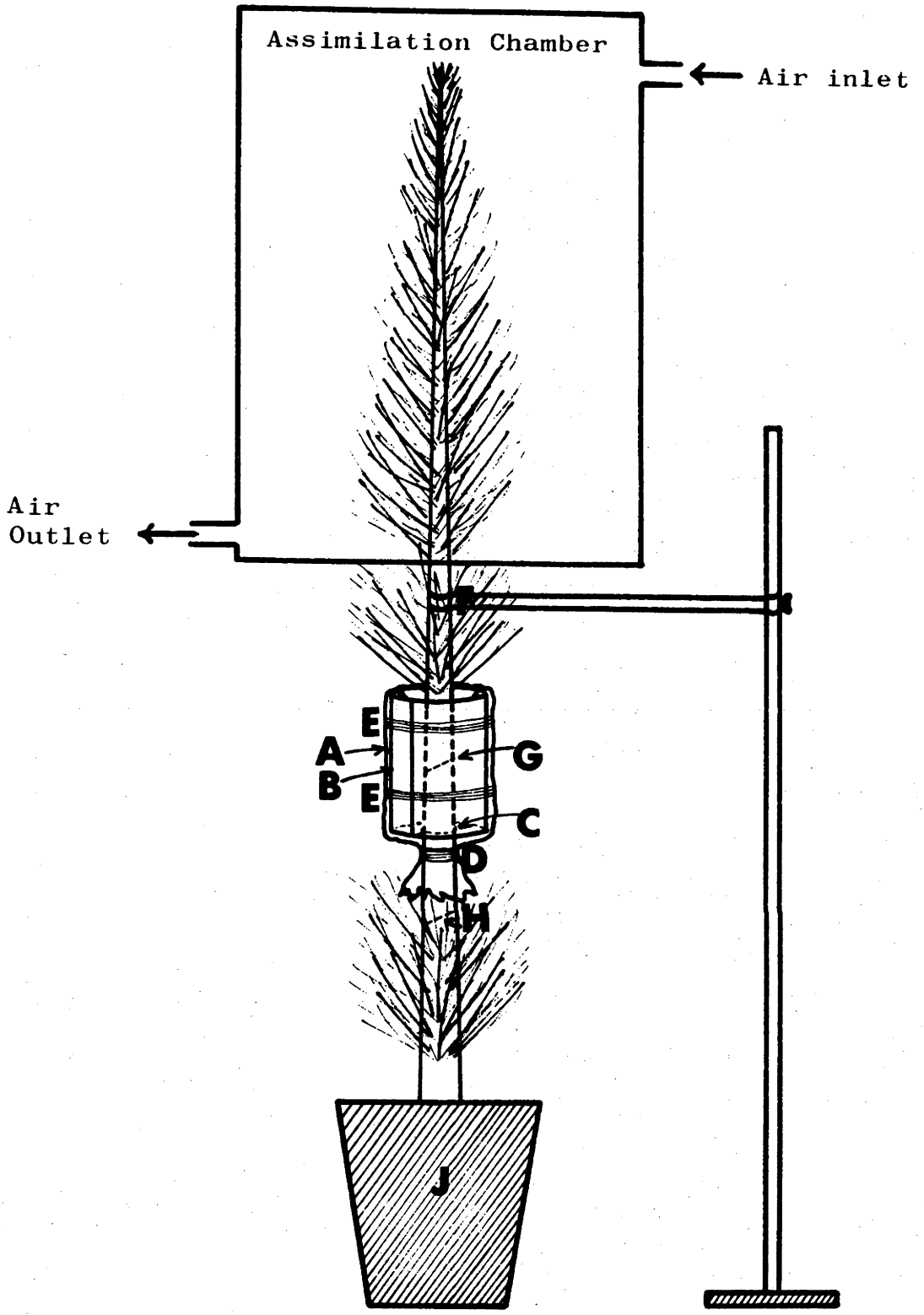


FIGURE 19 : Illustration of the technique used to sever the stem of an upright P. radiata seedling under water. See text for details.

2. The upper part of the shoot of the plant was sealed in the assimilation chamber at approximately 0900 hours and allowed one hour to re-acclimatise.

3. The rates of net P and transpiration were recorded at 1000 hours under the conditions of light and temperature previously noted.

4. Immediately Step 3 was completed, container B was filled with distilled water. The stem of the plant was then severed at point G under water using a sharp pair of secateurs. Then, holding the container in one hand, another cut was made at point H. The pot (J) containing the seedling stump was then removed and replaced by a large plastic beaker three quarters full of distilled water. This was positioned under container B and raised until the container and the base of the shoot were completely submerged. Container B was then allowed to sink to the bottom of the beaker and a support was placed under the base of the beaker. This left the base of the cut shoot submerged in distilled water. The severed end of the shoot was then trimmed with a sharp scalpel. With practice, Step 4 could be completed within one minute of the completion of Step 3.

5. The rates of net P and transpiration were recorded continuously for the first hour after severance, and at hourly intervals thereafter until the seventh hour.

The procedure described above was repeated on each of five seedlings, one per day on each of five consecutive days.

The rates of exchange of each seedling with time after severance are expressed as a percentage of the pre-severance rates of exchange.

RESULTS

Experiment 1

The rate of net P in both Treatments B and C was stimulated by treatment, that of the former possibly to a slightly greater extent than that of the latter (Figure 20). As some terminal shoot growth was evident in Treatments A and C at the end of the experiment, it is possible that some needle extension occurred during the experiment. This would cause the values of net P in the earlier phases to be underestimated.

Experiment 2

A stimulation of net P with time after decapitation is evident in both treatments (D and E) particularly at the higher levels of illumination (Table 12, Figure 21). The transpiration response, on the other hand, is too erratic to indicate the presence of any definite trend (Figure 22). The limited data presented in Table 13 suggest that one effect of treatment (at least in the case of Treatment E) was to increase the capacity of the photochemical partial process of photosynthesis.

Experiment 3

The responses of both net P and transpiration to severance were rapid (Table 14, Figure 23). The pattern of response of each was characterised by an

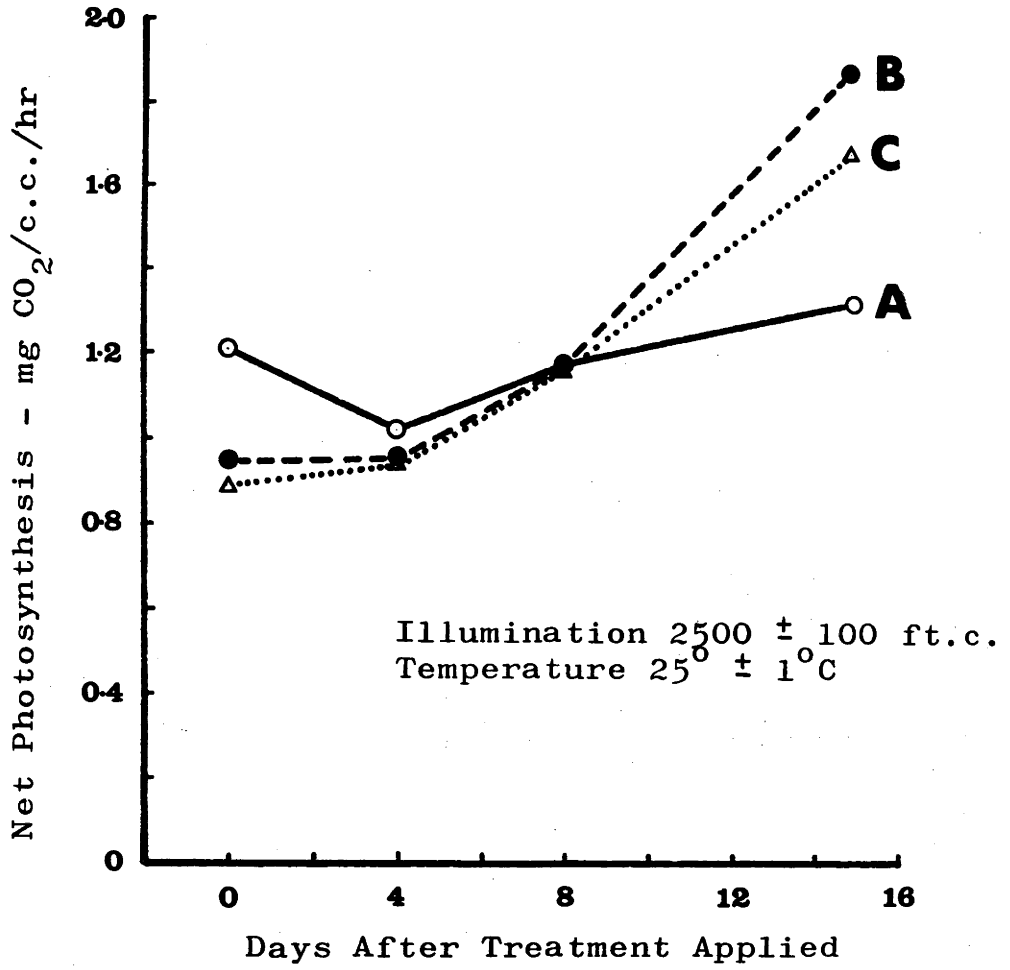


FIGURE **20**: Effect of severance of the apical shoot and/or removal of terminal foliage on the rate of photosynthesis of P. radiata seedlings.

A : Control treatment - terminal 20 cm of shoot enclosed in assimilation chamber.

B : Terminal 12 cm of shoot severed. 8 cm enclosed in chamber.

C: Terminal 20 cm of shoot enclosed in chamber. Foliage removed from the upper 12 cm section leaving the terminal rosette of foliage and bud intact.

TABLE 12 Time course of the rates of net photosynthesis (mg CO_2/c.c./hr) and transpiration (mg H_2O/c.c./hr) in foliage of P. radiata seedlings severed at the 85 per cent and 66 per cent levels of stem height (one seedling only per treatment)

Parameter and treatment	Days after severance	Illumination (ft.c.)				
		0	500	1000	2000	3000
Rate of CO_2-exchange	0	-0.23	0.25	0.71	1.32	1.56
	3	-0.28	0.22	0.62	1.16	1.51
	7	-0.26	0.32	0.90	1.68	1.95
D	11	-0.22	0.30	0.82	1.67	2.05
(85% level)	17	-0.26	0.24	0.81	1.74	2.14
Rate of CO_2-exchange	0	-0.21	0.34	0.91	1.67	1.86
	3	-0.30	0.29	0.80	1.59	1.90
	7	-0.27	0.36	0.99	1.90	2.26
E	11	-0.30	0.37	1.05	1.98	2.37
(66% level)	17	-0.29	0.36	1.01	1.98	2.47
Rate of transpiration	0	*	63	135	185	213
	3		25	77	122	160
	7		34	102	158	194
D	11		57	131	206	260
(85% level)	17		25	98	158	200
Rate of transpiration	0	*	120	179	238	264
	3		80	121	176	214
	7		142	170	215	260
E	11		93	162	235	303
(66% level)	17		75	131	191	248

* Data for the rate of transpiration in the dark are not presented as water loss continued to fall with time and did not stabilise

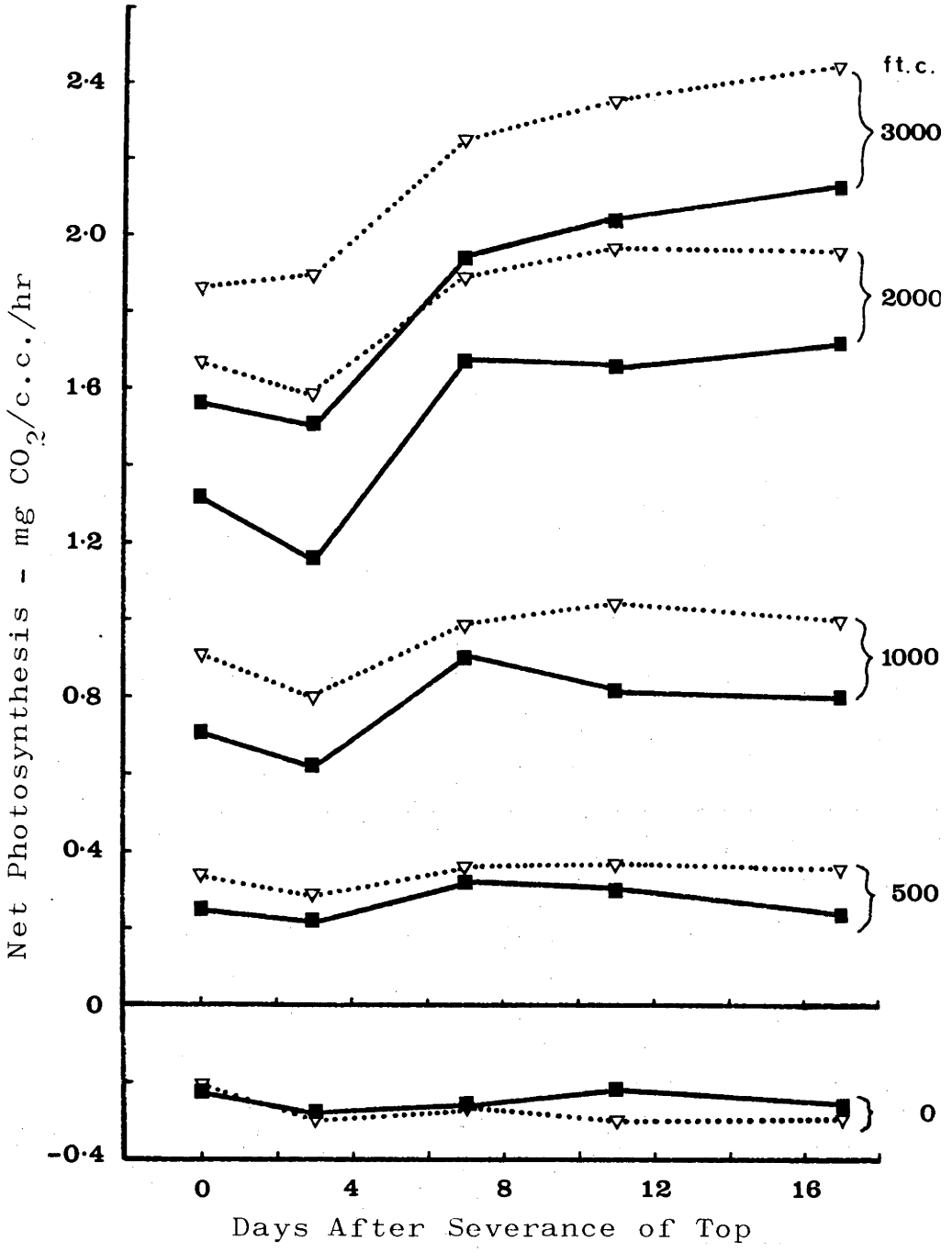


FIGURE 21: Effect of severance of the terminal shoot of P. radiata seedlings on the rate of net photosynthesis of foliage remaining on the stem.

■——■ Treatment D - Shoot severed at the 85% level of stem height.

▽·····▽ Treatment E - Shoot severed at the 66% level of stem height.

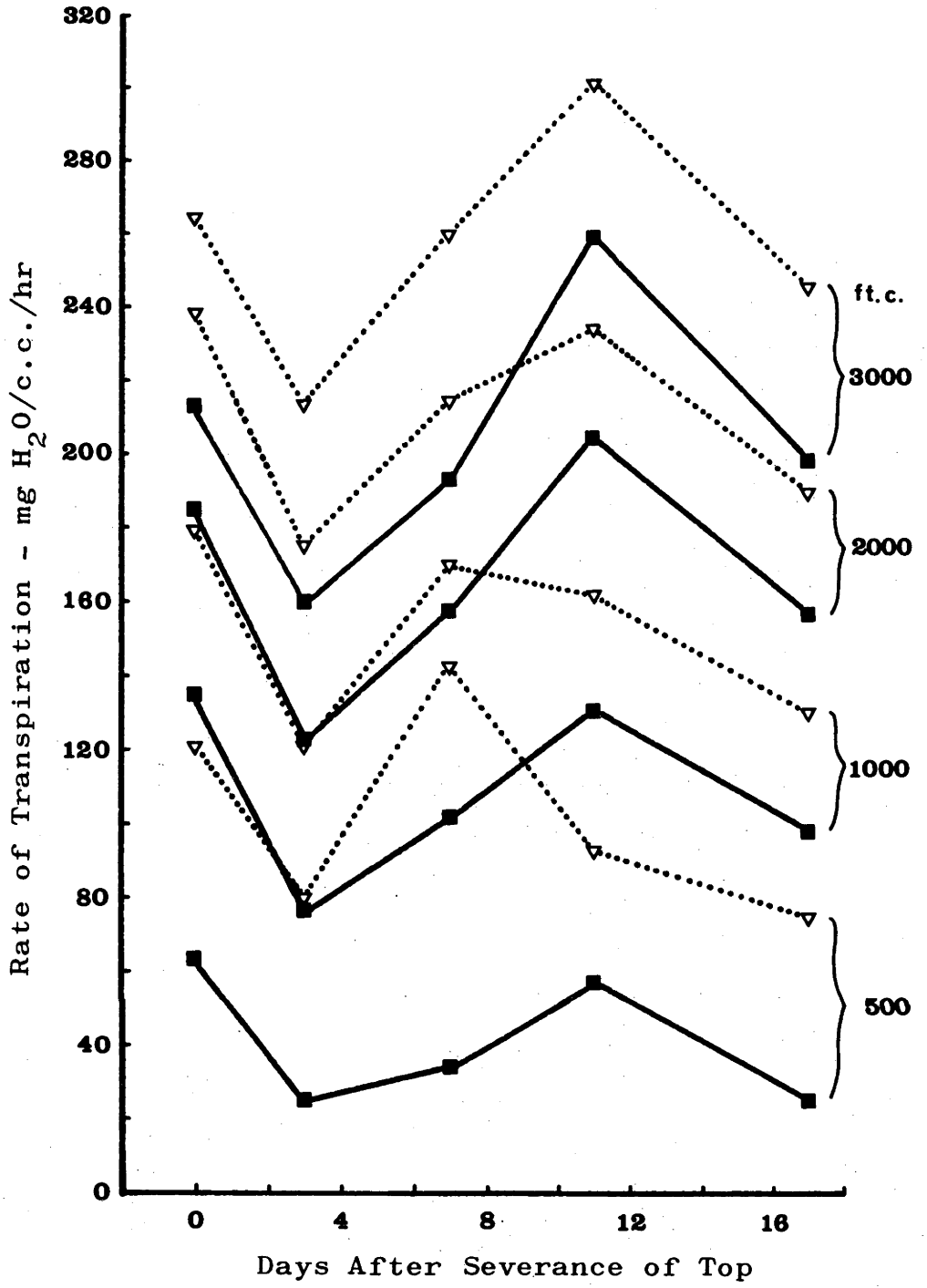


FIGURE 22: Effect of severance of the terminal shoot of P. radiata seedlings on the rate of transpiration of foliage remaining on the stem.

Treatment D - Shoot severed at the 85% level of stem height.

Treatment E - Shoot severed at the 66% level of stem height.

TABLE 13 Effect of time after severance of the terminal shoot on the initial slope of the light response curve for CO_2-exchange in P. radiata (one seedling only per treatment)

Days after severance	Treatment	Initial slope
0	D Severed at 85% level	2.4
3		2.5
7		2.9
11		2.6
17		2.5
0	E Severed at 66% level	2.8
3		3.0
7		3.2
11		3.4
17		3.3

TABLE 14 The effect of severance (under water) of the stems of P. radiata seedlings on the capacity of the shoots to photosynthesise and transpire. Each entry is an average of five replications

Time after severance (hours)	% of rate before severance		P/T ($\times 10^4$)
	Net photosynthesis	Transpiration	
0.00	100.0	100.0	824 ± 53
0.17	103.0 ± 2.3	113.5 ± 3.1	749 ± 50
0.33	104.8 ± 2.3	114.0 ± 1.8	761 ± 64
0.50	102.2 ± 1.2	112.8 ± 1.6	747 ± 50
0.67	102.3 ± 2.1	117.3 ± 3.0	719 ± 44
0.83	102.0 ± 2.9	123.7 ± 3.1	680 ± 47
1.00	104.6 ± 3.7	129.5 ± 3.9	666 ± 47
2.00	109.6 ± 3.7	136.5 ± 4.3	664 ± 53
3.00	107.1 ± 4.2	142.9 ± 7.5	617 ± 32
4.00	114.7 ± 7.6	157.8 ± 12.0	600 ± 35
5.00	117.5 ± 6.8	165.5 ± 13.6	586 ± 28
6.00	118.9 ± 6.0	167.6 ± 16.0	590 ± 33
7.00	116.5 ± 5.8	172.7 ± 17.3	561 ± 28

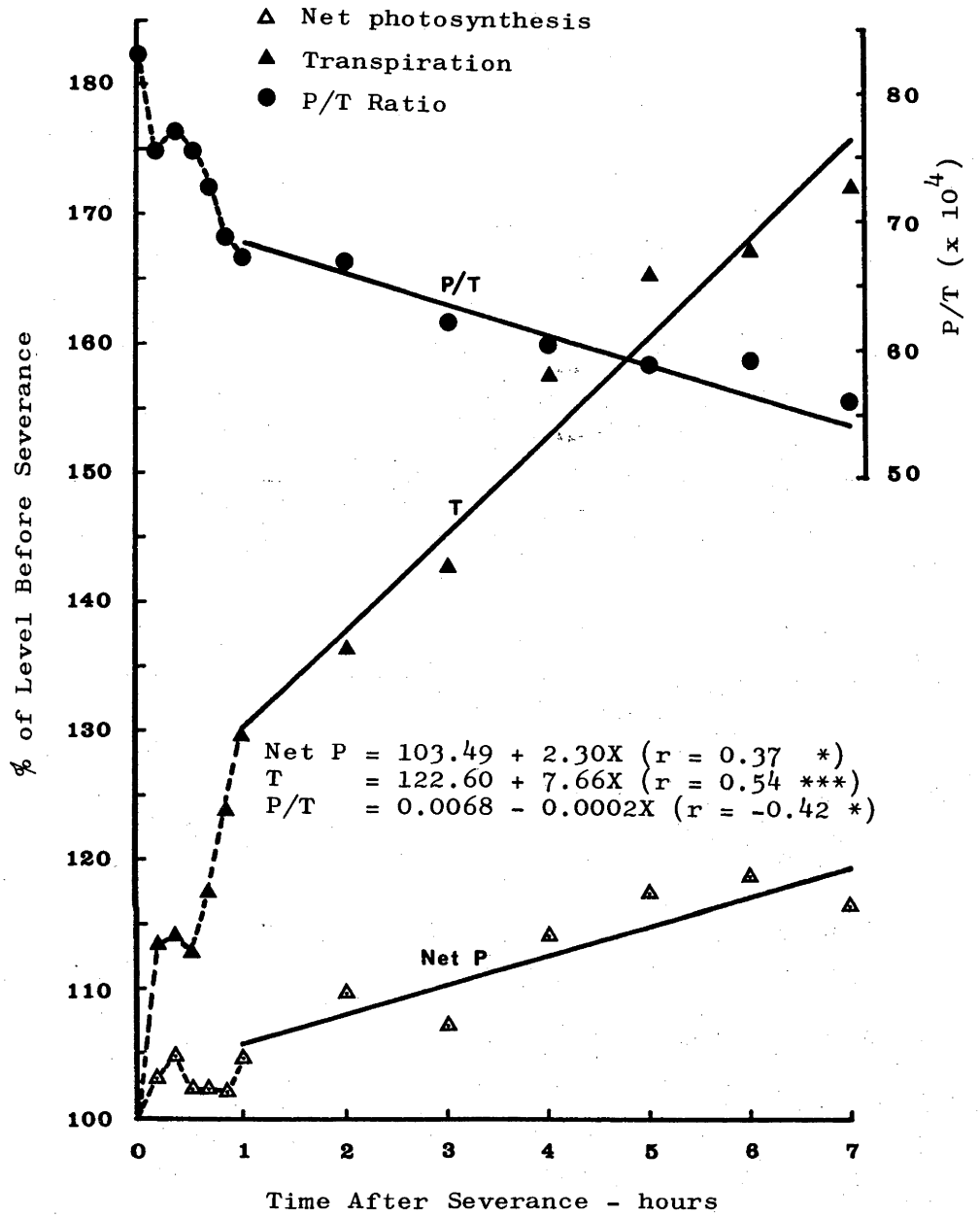


FIGURE 23: Effect of severance of the stem under water on the rates of net photosynthesis and transpiration and on the P/T ratio of shoots of P. radiata seedlings. Each point is a mean of five replicates.

initial surge followed by a period of adjustment lasting approximately 30 minutes. A gradual increase in the rate of each process with time then occurred, the increase in the rate of transpiration being much more marked than that in the rate of net P. Gas exchange in the final replicate was monitored continuously for 48 hours during which period the rate of each process continued to increase steadily with time.

DISCUSSION

One of the best known examples of a compensatory response in plants is where severance of leaves and shoots stimulates the growth of other plant parts. The stimulation of photosynthesis in P. radiata seedlings following decapitation (Figures 9, 20, and 21) is probably early evidence of such a growth response. As indicated previously (p. 34), compensatory responses form an interesting aspect of this work and will be considered in the general discussion.

The enhanced photosynthesis following removal of the apical portion of the stem or the young developing foliage (Figures 20 and 21) suggests that the presence of the young foliage and apical bud limited the photosynthetic capacity of older leaves. The change in the rate of photosynthesis induced by treatment may be due to a 'metabolic sink' effect (Sweet and Wareing, 1966), a disturbance of the plant hormonal balance (as has been demonstrated by Turner and Bidwell, 1965), or to the supply of nutrients and water being partitioned among fewer leaves with a consequent stimulation of their physiological activity (Hopkinson, 1966).

Whatever the causal factor, the absence of a parallelism in the effects on net P and transpiration suggests that the stomatal mechanism might not be involved.

The trend towards an increase with time in the initial slope of the light response curve for net P, at least in the mid-crown level (Table 13), suggests that an increased efficiency of the photochemical process may be partly responsible for the stimulation of net P observed with time. However, as the stimulating effect is greatest at higher light intensities (Figure 21), an increased efficiency of either the diffusion or biochemical process is principally involved (cf. Turner and Bidwell, op. cit.).

The higher rates of net P and transpiration in Treatment E than in Treatment D (Figures 21 and 22) may be to a large extent an expression of seedling variation. They could also be responses to treatment or an effect of foliage age. The photosynthetic capacity of foliage of conifers is known to increase in the first year of development until the foliage is mature (Freeland, 1952; Clark, 1961), and the data presented in Table 11 suggest that the foliage of Treatment D had not attained full maturity.

The stimulation of net P following decapitation in Experiment 1 (approximately 200 per cent increase in 15 days: Figure 20) is much higher than that observed at the same time after severance in Experiment 2 (130-140 per cent: Figure 21). The difference again may reflect seedling variation, but it could be

due to a seasonal effect. Experiment 1 was conducted in early summer when the seedlings were growing vigorously, whereas Experiment 2 was conducted in mid-winter when they were semi-dormant.

The gradual increase with time after severance in the capacity of the severed shoot to photosynthesise and transpire (Experiment 3: Figure 23) suggests that the stomatal width was increasing. The lesser effect observed on net P than on transpiration is consistent with this view, as the former process is less affected by a change in stomatal resistance than is the latter (Gaastra, 1959). A gradual increase in the stomatal width with time could occur due to a progressive lessening of the imbalance between transpiration loss and water absorption following the removal of the root system. This would lead to a gradual decrease in the leaf water deficit. One would expect an end to the stomatal opening process, however, on the removal of the imbalance. The fact that both net P and transpiration continued to increase with time after severance suggests that some other internal process was also operating. A progressive reduction in mesophyll resistance is discounted because such would stimulate net P more than transpiration, and this clearly did not occur. It is possible that hormonal change or a 'metabolic sink' effect is involved. Further investigation is warranted.

It is obvious from Figure 23 that the detached shoot technique described is useless for studying transpiration responses in P. radiata. On the other hand, a study of photosynthetic responses would appear

to be feasible provided it is made as soon as possible (preferably less than 4 hours) after shoot severance.

III FIELD STUDIES

In the previous section, the reaction of *P. radiata* seedlings to several environmental parameters was studied in the laboratory. In this section, some of the needle morphological features and physiological conditions existing within the crown of a plantation tree are examined and an assessment is made of the photosynthetic activity of foliage sampled from various positions in the crown. The data will be considered in the light of the laboratory findings.

MATERIALS AND METHODS - GENERAL

Plot location: The studies on the spatial distribution of foliage and on the spatial variation in foliage morphology and chlorophyll content within the crown (Studies 5, 6, and 7 respectively) were conducted in a small forest plot located in Uriarra State Forest, A.C.T. The plot was planted as part of the surrounding forest at 8' x 8' spacing in Winter 1959. At the time of study, the stand was 6-7 years of age, almost fully stocked, and just closing its canopy. The average height of dominants was approximately 25 feet. A plot of approximately equal development closer to Canberra was selected from which gas exchange studies could be made (Study 8). This plot was located in an unpruned 7-year-old 2-acre stand situated on the lower NE slope of Black Mountain about two miles from the A.N.U. laboratory. The stand was stocked at 500 stems

per acre and the height of dominants was 28 feet. The canopy was closing.

Study material: In each stand, study was confined to a single healthy tree of average height and diameter for the plot. Sampling of other trees, though desirable, was not possible because of time limitations.

Instrumentation, and measurement of plant parameters: The instrumentation for measuring CO_2-exchange and the techniques used to measure needle length, thickness, surface area, volume, pigment content, relative water content, etc., have been described previously. The annual growth stages on the main stem and branches of the study trees were detected by the method of Jacobs (1936). This enabled the chronological age of all foliage on each tree to be determined.

STUDY 5

SPATIAL DISTRIBUTION OF FOLIAGE IN THE CROWN OF A PLANTATION SAPLING

In a later field study, data are presented on the spatial variation in the photosynthetic activity of needles within the crown of a plantation tree. This information will be of limited value in assessing the probable importance of various sections of the crown to the growth of the tree without access to data on the relative quantities and spatial distribution of needles of different ages within the crown. The study described below was an attempt to gather some relevant information for a 6-year-old plantation tree.

METHODS

The height above ground of each branch whorl on the tree was measured. The foliage was then systematically stripped from the tree commencing with the one-year-old foliage on each branch of the basal whorl. Two-, three-, four-, and five-year-old needles were then stripped in sequence until all the branches of the whorl were bare of foliage. The foliage was sealed in plastic bags by ages and labelled. The process was repeated on each of the 15 branch whorls on the tree until all branches were bare of foliage. Finally, the stem foliage was stripped from the bole and bagged by ages. All the material was then taken to the laboratory, oven dried, and weighed.

RESULTS

Results are presented in Table 15 and illustrated in Figures 24 and 25. These indicate:

(i) 1-year-old foliage occurs at all levels in the crown but is concentrated above the 25 per cent level (the percentage level is the height of a point in the crown above ground expressed as a percentage of total stem height).

(ii) 2-year-old foliage is confined largely to the lower half of the crown and is concentrated between the 20-50 per cent levels.

(iii) 3- and 4-year-old foliage is confined to the lower crown, occurring below the 40 per cent and 30 per cent levels respectively.

(iv) 5-year-old needles occur near ground level but their quantity is insignificant.

(v) The greatest concentration of foliage on the tree (all ages) occurs between the 25-50 per cent levels.

(vi) Stem fascicles are concentrated in the upper half of the crown, but while their absolute quantity is small (approximately 4 per cent of the total weight of foliage on the tree) they occur in the region of the crown where the concentration of branch foliage is comparatively low.

(vii) The absolute quantities of 1- and 2-year-old foliage on the tree are roughly comparable, and together they comprise more than 80 per cent of the total dry weight of foliage.

TABLE 15: Distribution of foliage (g O.D.Wt.) on an age, height, and whorl basis over the crown of a 6-year-old P. radiata plantation tree.

Whorl no.	Whorl height (ft)	Season whorl developed Δ	Foliage age (years) 1	2	3	4	5	Weight per whorl (g)	% of total weight of foliage on tree
1	0.8	1960/61	2	19	5	28	-	54	1.0
2	1.0		10	36	6	38	-	90	1.7
3	1.6		34	110	20	130	-	294	5.6
4	1.8		55	197	54	208	6	520	9.8
Total on 1960/61 whorls			101	362	85	404	6	958	18.1
5	3.3	1961/62	47	90	10	36	-	183	3.5
6	3.8		92	214	28	86	-	420	7.9
7	4.4		231	365	60	110	-	766	14.5
Total on 1961/62 whorls			370	669	98	232	-	1369	25.9
8	5.3	1962/63	228	351	99	-	-	678	12.8
8-9*	5.3-6.9		-	-	7	-	-	7	0.1
9	6.9		165	170	17	-	-	352	6.7
9-10*	6.9-7.9		-	-	4	-	-	4	0.1
Total on 1962/63 whorls			393	521	127	-	-	1041	19.7
10	7.9	1963/64	521	371	-	-	-	892	16.9
10-11*	7.9-8.5		-	21	-	-	-	21	0.4
11	8.5		80	35	-	-	-	115	2.2
11-12*	8.5-10.7		-	44	-	-	-	44	0.8
Total on 1963/64 whorls			601	471	-	-	-	1072	20.3
12	10.7	1964/65	374	-	-	-	-	374	7.1
12-13*	10.7-12.0		17	-	-	-	-	17	0.3
13	12.0		264	-	-	-	-	264	5.0
13-14*	12.0-13.2		31	-	-	-	-	31	0.6
14	13.2		75	-	-	-	-	75	1.4
14-15*	13.2-14.9		50	-	-	-	-	50	1.0
15	14.9		12	-	-	-	-	12	0.2
15-16*	14.9-15.7		16	-	-	-	-	16	0.3
16-Tip*	15.7-16.2 ∅		5	-	-	-	-	5	0.1
Total on 1964/65 whorls			844	-	-	-	-	844	16.0
Grand total for tree			2309	2023	310	636	6	5284	100.0
% of total by ages			43.7	38.3	5.9	12.0	0.1		

Δ The 1960/61 season is from winter 1960 to winter 1961, etc.

* Refers to stem foliage between whorls

∅ The resting bud (16.2'-17.5') was devoid of foliage.

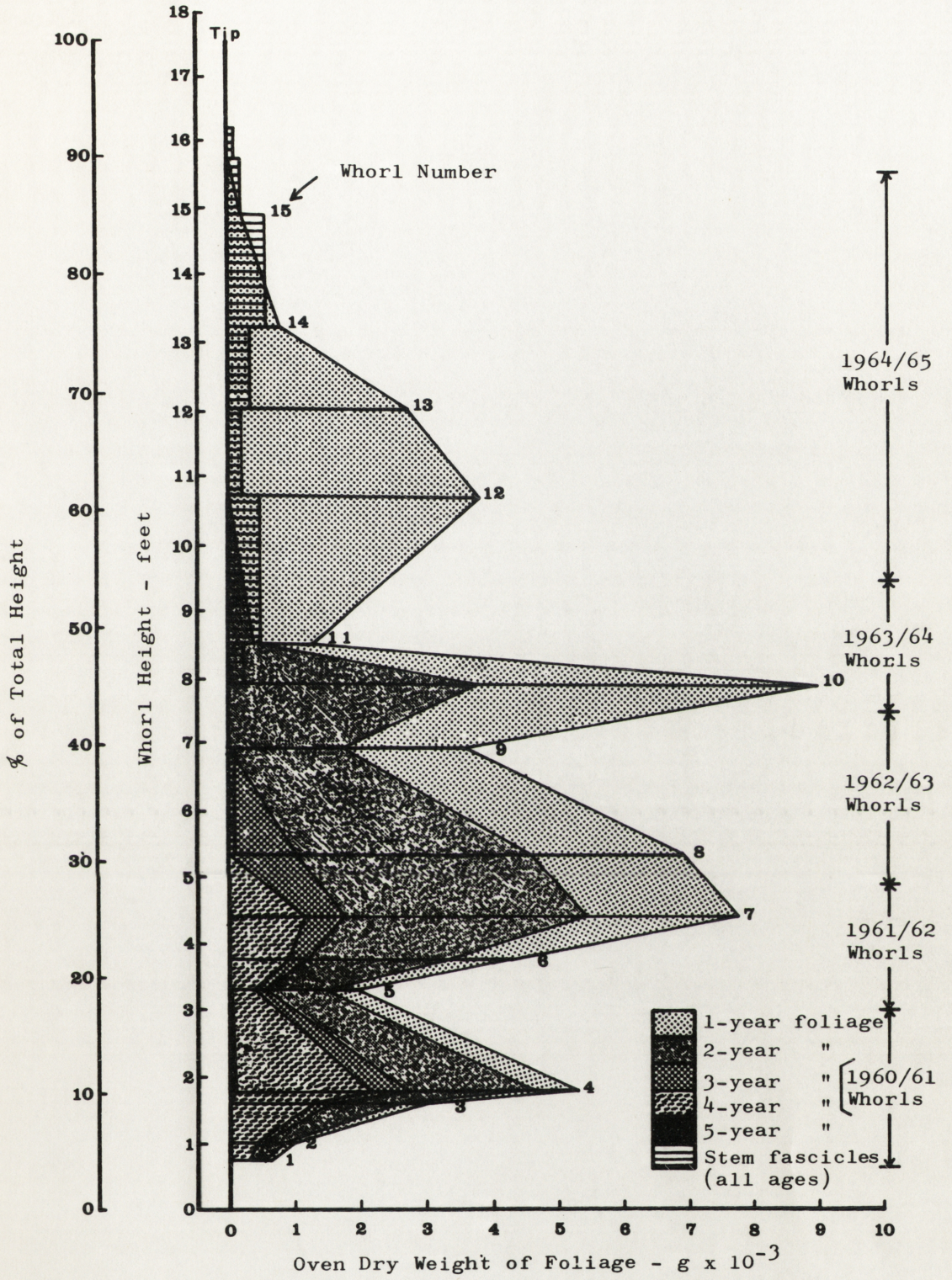


FIGURE 24 : Vertical distribution of foliage by ages within whorls in the crown of a 6-year-old P. radiata tree 17½ feet tall.

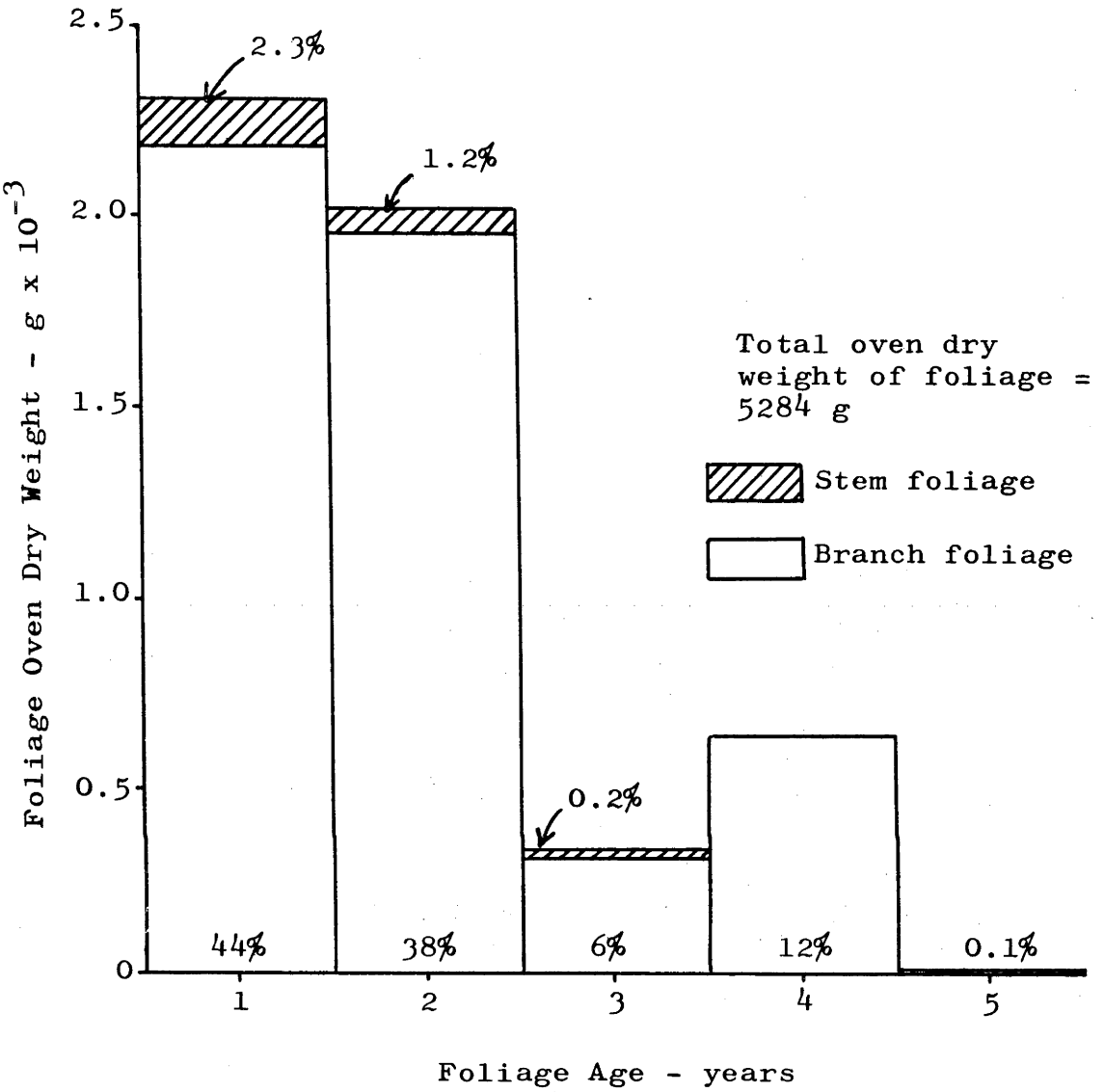


FIGURE **25** : Distribution of foliage by age classes in the crown of a 6-year-old P. radiata stem 17½ feet tall.

DISCUSSION

The spatial distribution of foliage of various ages within the crown (Figure 24) fits the usual pattern for *Pinus* species, viz., the younger leaves predominate in the upper crown and the older leaves in the lower crown, while the maximum concentration of foliage of all ages occurs in the mid-crown region (cf. Hall, 1966). The concentration of older foliage at the base of the crown is partly due to crown recession and partly to the persistence of basal foliage. The observation that *P. radiata* foliage can persist for as long as five years has also been made by Haasis (1931) and Will (1957).

There appear to be few published data on the relative contribution of needles of different ages to the total weight of foliage in the crown of *Pinus* species. Reference to Madgwick (1962) and White (1964) indicates in 30-year-old *P. resinosa* that 1- and 2-year-old needles predominate and together they constitute approximately 80 per cent of the total foliage weight. These data are comparable with the data of this study (Figure 25).

The presence of stem foliage in the upper crown levels, where the concentration of branch foliage is relatively low, is particularly significant. This foliage, while small in quantity, is concentrated in the regions of the crown receiving abundant light. It is expected that it would contribute to the photosynthetic efficiency of trees during the juvenile, adolescent, and bulbous stages of growth (see Jacobs,

1936) which are characterised by few branches and/or a long terminal spike.

The results presented are indicative only because only one tree was examined and so many factors determine the distribution of foliage of various ages within the crown of a tree, e.g., tree size and age, stand density, site quality, insect damage and disease, unfavourable weather conditions, flowering, etc. (Clark, 1961; Metz and Wells, 1965). Despite this, it is believed that the data illustrate the type of distribution pattern of foliage one might expect to find in the crown of a 6-year-old P. radiata plantation tree.

STUDY 6

SPATIAL VARIATION IN FOLIAGE MORPHOLOGY WITHIN THE CROWN OF A PLANTATION SAPLING

The marked variation in the light gradient within forest stands affects leaf development, as is well known from the results of research conducted during the late nineteenth and early twentieth centuries (see Büsgen and Munch, 1929; Helmers, 1943). However, it is incorrect to presume, as is sometimes done (e.g., Clark, 1961; Shakhov et al., 1965), that the variation in foliage characteristics with height in the canopy is due entirely to variation in the light climate under which the foliage was produced. Some of the variation may be accounted for by ontogenetic differences and by the distribution patterns of moisture, nutrients, and of plant hormones within the crown (Granick, 1949; Will, 1957; Sinnott, 1960).

Evidence suggests that a marked reduction in light intensity occurs from the top to the base of the crown of a young plantation tree of P. radiata (Appendix 10, Table A3). As the development and photosynthetic capacity of foliage of P. radiata seedlings in the laboratory was greatly affected by the light climate under which the seedlings were grown (Study 2), interpretation of the results of the final field study, which investigates the photosynthetic activity of foliage at different levels in the canopy, possibly will require some knowledge of the spatial

variation in needle morphology within the canopy (see Weir et al., 1964).

METHODS

Sampling procedure: The spring whorls which developed in the years 1960-4 inclusive were sampled. Sampling within a whorl was confined to foliage on the first order branches facing approximately north and south respectively. Foliage of all ages on these branches was sampled unless the quantity of a particular age was too small to permit sampling. Each sample comprised 21 ternate fascicles which were detached from all sides of the branch at the middle of the annual shoot. The height of the point of attachment above ground was recorded. Samples were labelled, bagged, and taken to the laboratory. Three fascicles of each sample were preserved in F.A.A. for subsequent anatomical examination.

Measurement procedure: Measurements of various needle parameters were made on sub-samples of needles using techniques previously described. Thirty needles were used for the measurement of needle length, thickness, surface area, and fascicle sheath length; fifteen for a count of the number of stomatal lines on the abaxial face of a needle; and nine for determining needle moisture content and the dry weight of foliage. The dry weight per 1000 needles was then determined by proportion.

Computational procedure: Foliage was sampled from the north and south sides of the tree because the

morphological characteristics of Pinus needles have been reported to vary with orientation about the bole at any one horizontal level within a tree crown (see Helmers, 1943). Inspection of the data reveals, however, that the effect of orientation on the characteristics of foliage in the young tree studied was negligible when compared with the effects of needle age and height of needle insertion in the canopy (Table 17). Thus, when analysing the data, the effects of orientation about the bole were ignored.

Plotting the sets of data presented in Table 16 suggests curvilinear trends of a quadratic nature (see Ezekiel, 1941) largely controlled by the variables of needle age and height of insertion in the canopy (Figure 26). Consequently, the data were subjected to multiple regression analysis based on the model:

$$Y = a_1 + a_2B + a_3B^2 + a_4C + a_5C^2 + a_6BC$$

where Y is the dependent variable

B is needle age

C is the height of needle insertion in the crown

a_1 is the regression constant

and a_2, a_3, a_4, a_5, and a_6 are the regression coefficients.

Because of the finding in the laboratory that the number of stomatal lines per mm of needle thickness was unaffected by the light climate under which seedlings were grown (Appendix 5, Table A11), the data

TABLE 16: Spatial variation in some of the morphological features of foliage in the crown of a 6-year-old P. radiata plantation tree.

Legend: (1) Sample no. (2) Orientation (3) Needle age-years (4) Sample height - ft (5) Needle thickness - mm (6) Needle length - cm (7) Needle surface area - cm^2 (8) Fascicle sheath length - cm (9) O.D.Wt. of foliage/1000 needles - g (10) No. of stomatal lines/needle-abaxial face (11) Needle moisture content - %.

1	2	3	4	5	6	7	8	9	10	11
1	NE	1	2.5	0.40	3.72	0.60	2.3	2.4	4.4	150
5	NE	1	4.0	0.53	6.94	1.47	4.1	7.9	5.6	156
8	S	1	4.0	0.45	4.03	0.73	2.4	3.1	4.1	144
14	SW	1	6.0	0.70	9.10	2.55	5.8	15.3	7.3	150
11	NE	1	8.0	0.70	9.52	2.67	5.6	17.2	7.5	157
19	SSW	1	9.0	0.79	10.01	3.16	5.9	21.9	7.9	145
17	NE	1	10.5	0.86	10.33	3.55	6.2	25.8	8.8	144
22	SSE	1	13.5	0.90	12.35	4.45	7.6	36.1	10.3	156
21	NNE	1	14.0	0.89	12.25	4.36	7.0	32.8	10.0	146
2	NE	2	2.0	0.57	6.64	1.51	2.5	7.0	5.3	137
4	SSE	2	2.0	0.52	7.94	1.65	2.1	7.6	4.8	161
6	NE	2	3.3	0.62	9.20	2.28	3.1	13.0	6.8	141
9	S	2	3.3	0.66	10.11	2.67	3.8	15.5	7.2	151
15	SW	2	5.0	0.79	11.88	3.75	4.0	25.4	7.7	130
12	NE	2	6.3	0.76	12.38	3.76	4.5	28.6	7.9	133
20	SSW	2	7.3	0.86	13.53	4.65	3.4	40.5	9.7	134
18	NE	2	8.5	0.91	13.95	5.08	4.2	43.6	9.4	133
7	NE	3	2.8	0.66	11.13	2.94	shed	21.7	7.9	133
16	SW	3	4.5	0.76	12.36	3.76	shed	22.9	7.6	122
13	NE	3	5.5	0.85	12.42	4.22	shed	30.1	7.6	140
3	NE	4	1.5	0.66	10.12	2.67	shed	14.1	7.2	143
10	S	4	2.8	0.69	11.40	3.15	shed	23.9	8.2	144
23	Stem fascicles	1	15.0	1.07	15.16	6.49	7.0	64.5	13.1	143
24		2	9.5	1.18	16.67	7.86	4.8	70.7	14.0	126
25		3	6.0	0.87	14.24	4.96	shed	55.3	8.9	139

TABLE 17: Results of the multiple regression analyses using various needle parameters as the dependent variable (Y), and needle age in years (B) and height of insertion in the canopy in feet (C) as the independent variables

Dependent variable	Regression equation Δ	Correlation coefficient (r)	Significance of regression ø
Needle thickness (mm)	$Y = -0.15 + 3.353B - 0.045B^2 + 0.113C - 0.004C^2 - 0.011BC$	0.98	***
Needle length (cm)	$Y = -8.19 + 8.917B - 1.192B^2 + 2.009C - 0.064C^2 - 0.213BC$	0.97	***
Needle surface area (cm^2)	$Y = -3.24 + 2.545B - 0.345B^2 + 0.617C - 0.018C^2 + 0.025BC$	0.98	***
Fascicle sheath length (cm)	$Y = -2.4 + 3.061B - 0.590B^2 + 1.511C - 0.038C^2 - 0.473BC$	0.95	***
Oven dry weight (g)/ 1000 needles	$Y = -21.3 + 13.131B - 1.906B^2 + 3.272C - 0.088C^2 + 1.181BC$	0.96	***
No. stomatal lines on abaxial face	$Y = -1.7 + 3.694B - 0.404B^2 + 1.176C - 0.028C^2 - 0.170BC$	0.94	***
Needle moisture content (%)	$Y = 175.5 - 18.004B + 3.061B^2 - 2.173C + 0.155C^2 - 0.703BC$	0.72	*

ø * indicates significance at $p<.05$

*** indicates significance at $p<.001$

Δ Some terms in the equations presented were non-significant based on the t-test. However, they are not excluded from the equations presented because with additional samples they could all attain significance (based on advice of G.A. McIntyre, Division of Mathematical Statistics, C.S.I.R.O.).

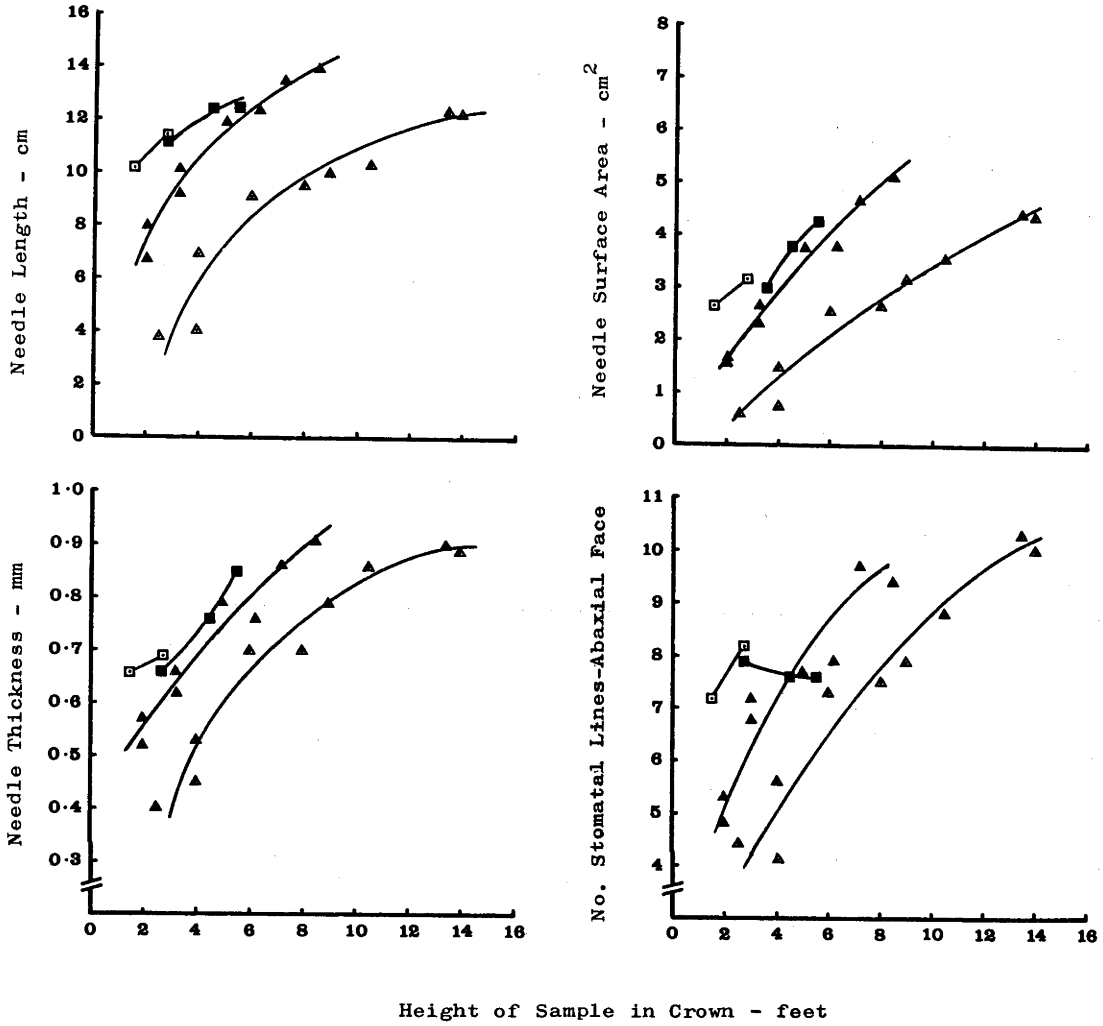


FIGURE 26: Variation in needle morphology with age and height of insertion in the crown of a 6-year-old P. radiata plantation tree

1-year needles

2- " "

3- " "

4- " "

of the current study were examined to determine whether a similar type of relationship between needle thickness and number of stomatal lines exists in the crown of a plantation tree.

RESULTS

Results of the multiple regression analyses are summarised in Table 17. With the exception of needle moisture content, all the characteristics of the needles investigated were highly correlated with needle chronological age and height of insertion in the crown, the regressions in all cases being significant at $p < .001$. Needle thickness, length, surface area, oven dry weight, and the number of stomatal lines per needle all increased with increase in needle age and height of insertion in the canopy. Fascicle sheath length increased with height but decreased with age, the sheaths shedding in the third year. The regression of needle moisture content on age was significant at $p < .05$. Needle moisture content tended to decrease with needle age but the trend is poorly defined. For a given age, it was unaffected by the height of needle insertion in the canopy.

The relationship between needle thickness and the number of stomatal lines on the abaxial face is linear (Figure 27), the solution being:

$$Y = -0.33 + 10.98\ X \qquad (r = 0.94^{***})$$

where Y is the number of stomatal lines

and X is needle thickness in mm.

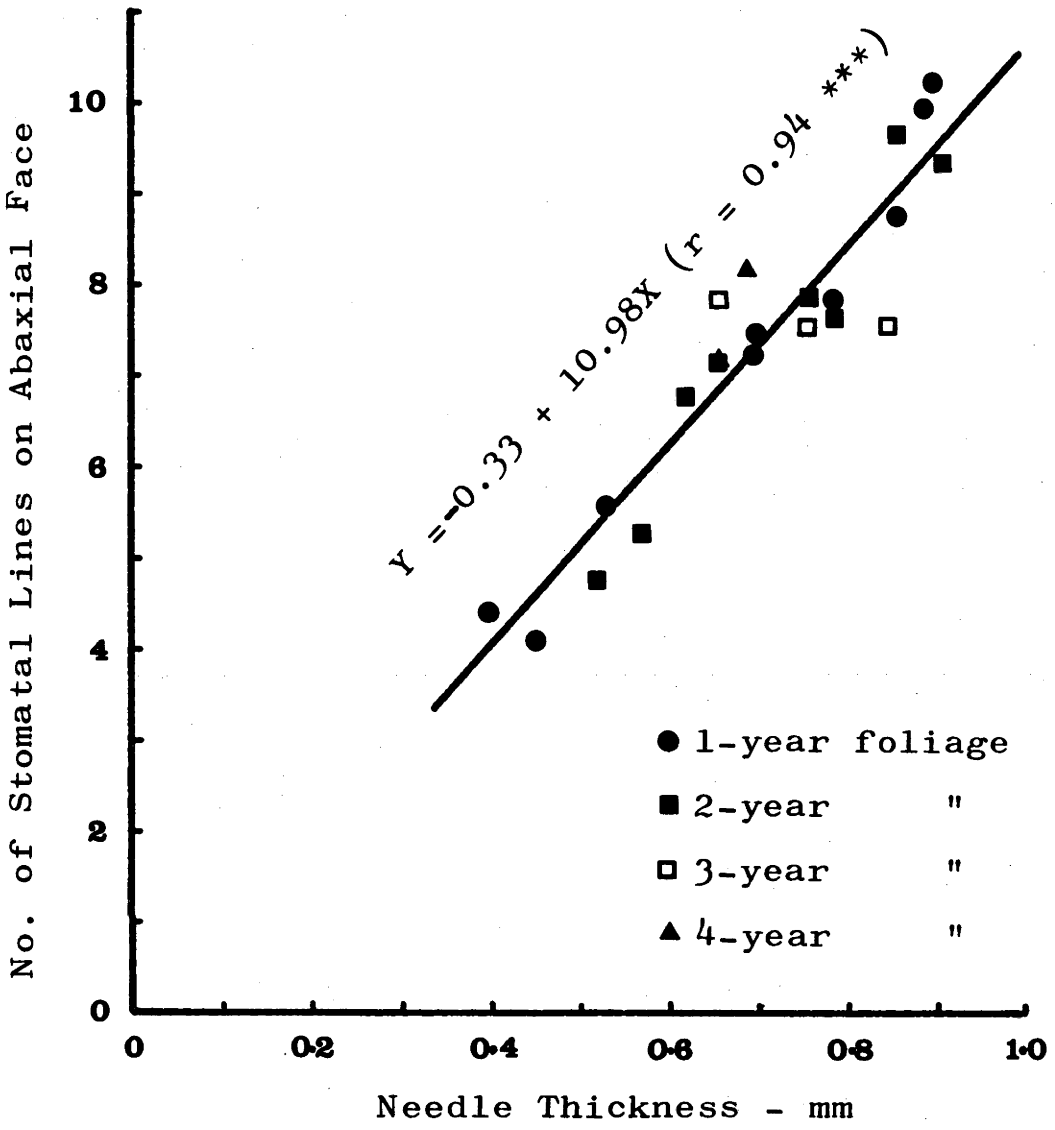


FIGURE 27 : Relationship between needle thickness and the number of stomatal lines on the abaxial face of P. radiata needles sampled from all levels of the crown of a 6-year-old plantation tree.

A curvilinear relationship exists between needle oven dry weight and surface area irrespective of needle age and of the height of needle insertion in the canopy (Figure 28). This suggests that it may be possible to estimate leaf surface area from a measurement of leaf dry weight. A proviso would be that the number of needles per fascicle should not vary, as a change in fascicle order implies a change in the basic shape of the needle and in the surface area/weight ratio.

Examination of transverse sections of needles sampled from different parts of the tree crown (see Plates 6-8) indicates that, irrespective of crown position, the epidermal, mesophyll, and vascular tissues form a constant proportion of the total leaf cross-sectional area, the proportions being .16 $\pm$ .01, .50 $\pm$ .01, and .34 $\pm$ .01 respectively. These proportions are almost identical with those presented earlier for the foliage of seedlings grown under a wide range of conditions in the 'CERES' phytotron (p.33).

While the basic structure of the needle is unaltered by crown position, the physical size of needles differs appreciably, e.g., needles in the upper crown are larger and have a better developed vascular system and more palisade and sponge mesophyll tissue than needles of the same age in the lower crown (Plate 6). Similar differences are apparent when old needles are compared with young needles inserted at the same height in the crown (Plate 7). There is some evidence of a disintegration and disorganisation of the palisade and sponge mesophyll tissue in 3-year-old foliage

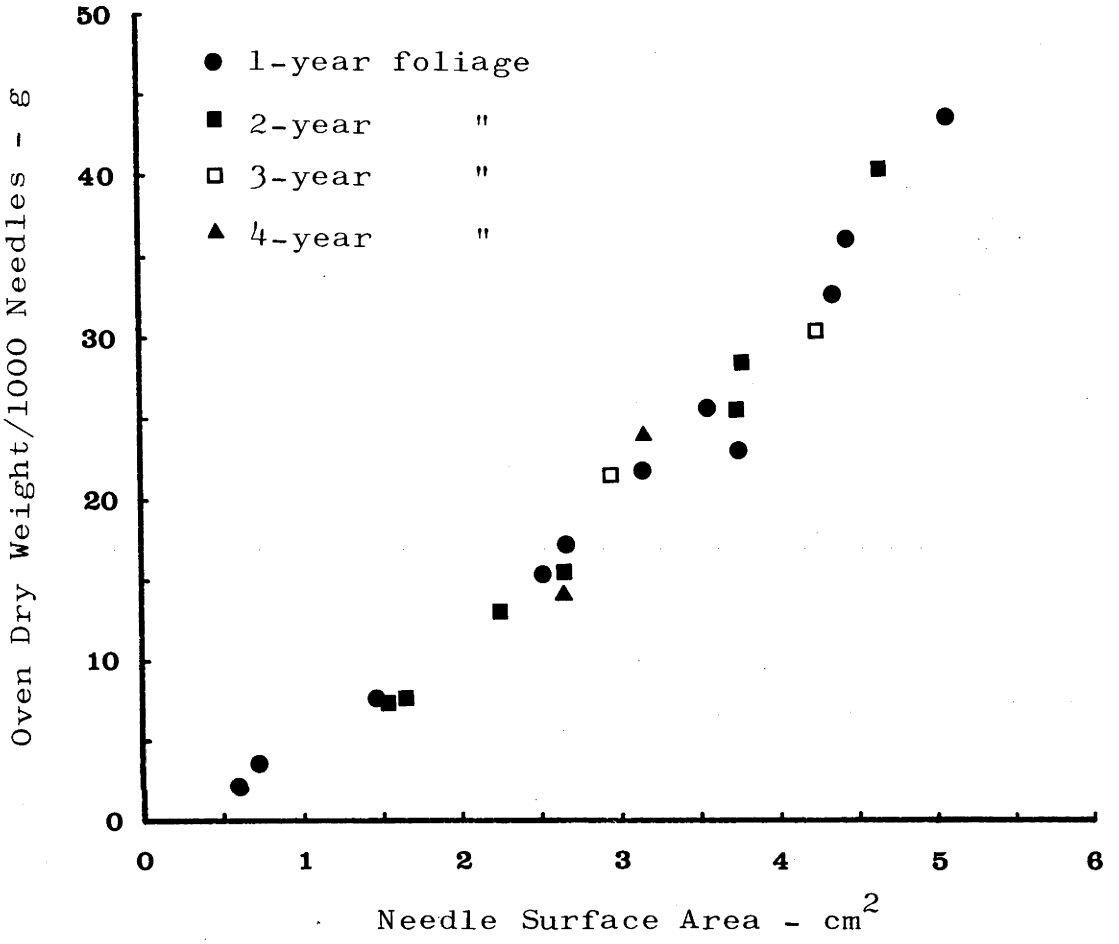


FIGURE **28 :** Relationship between surface area and dry weight in P. radiata needles sampled from all levels of the crown of a 6-year-old plantation tree.

PLATE 6 Variation in the anatomical structure of 1-year-old P. radiata needles inserted at different heights in the crown of a 6-year-old plantation tree.

Upper section : 4' above ground
Middle section : 8' above ground
Lower section : 13½' above ground

Sections photographed under low power at a magnification of 50X.

Each minor scale division represents 0.01 mm.

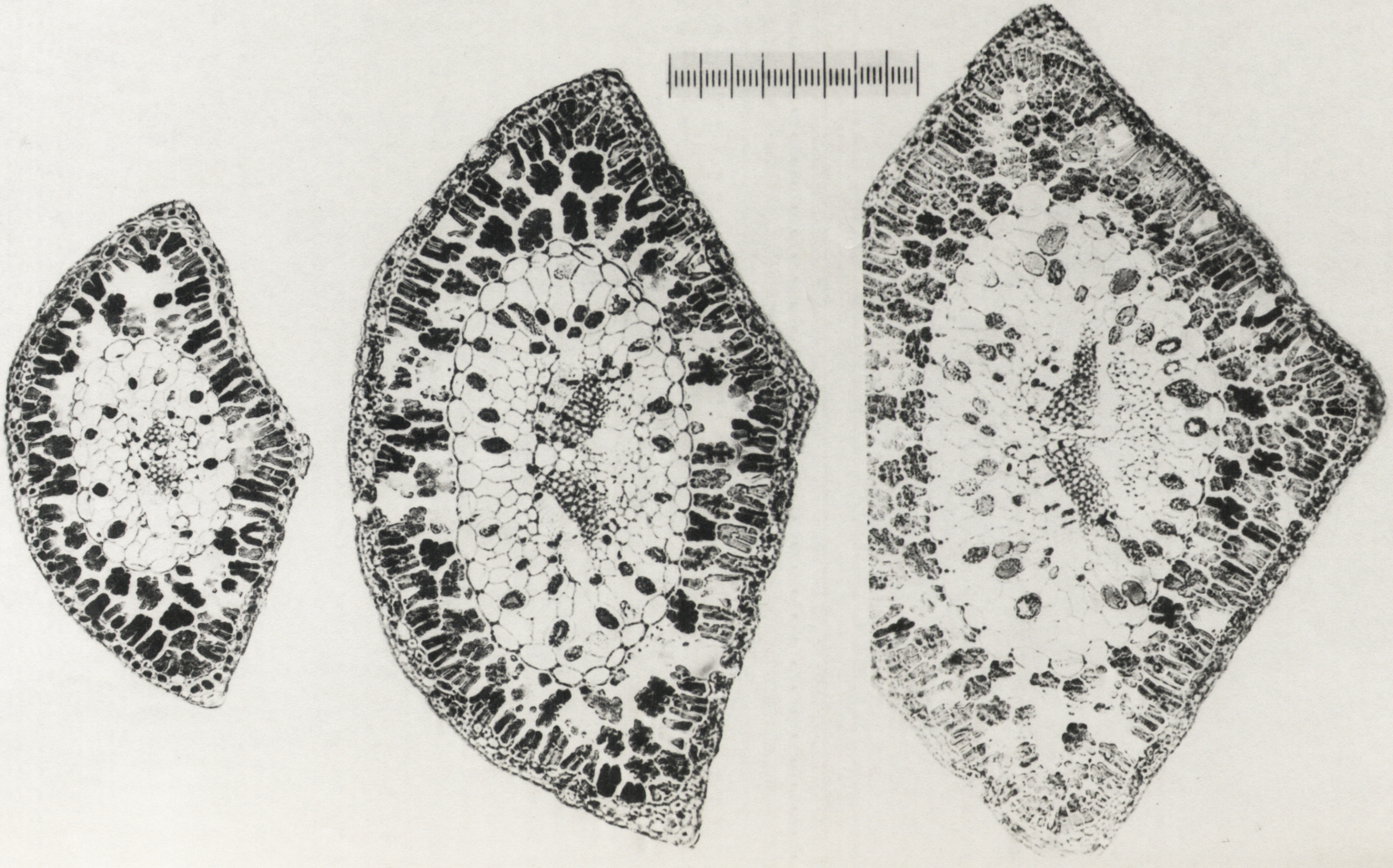


PLATE 7 Variation in the anatomical structure of *P. radiata* needles of different ages inserted at approximately the same height in the crown of a 6-year-old plantation tree.

Upper section : 1-year-old foliage 4' above ground

Middle section : 2-year-old foliage 5' above ground

Lower section : 3-year-old foliage $4\frac{1}{2}$' above ground

Sections were photographed under low power at a magnification of 50X.

Each minor scale division represents 0.01 mm.

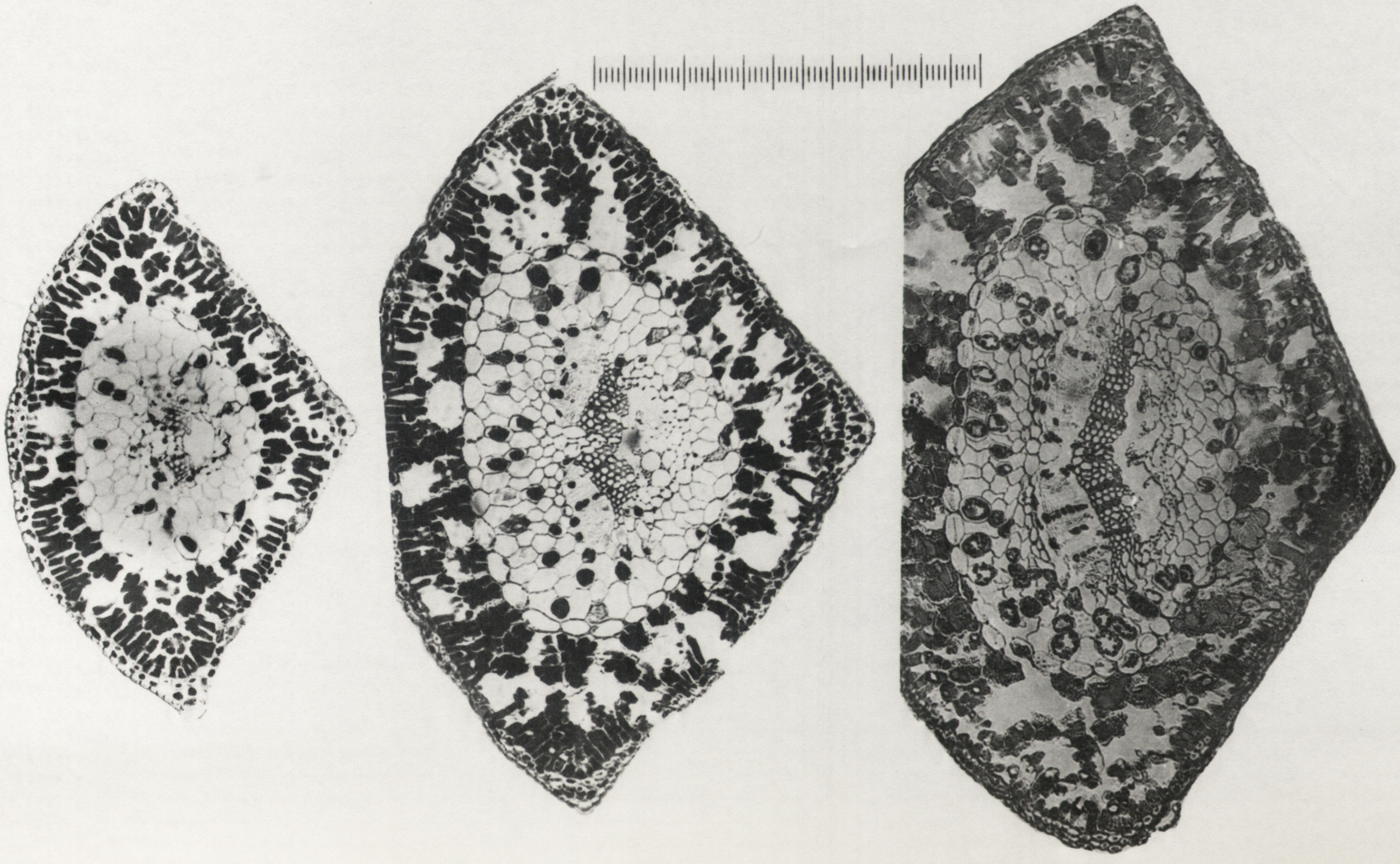


PLATE 8 Variation in the anatomical structure of P. radiata needles, sampled from stem fascicles of various ages inserted at various heights above ground in the crown of a 6-year-old plantation tree.

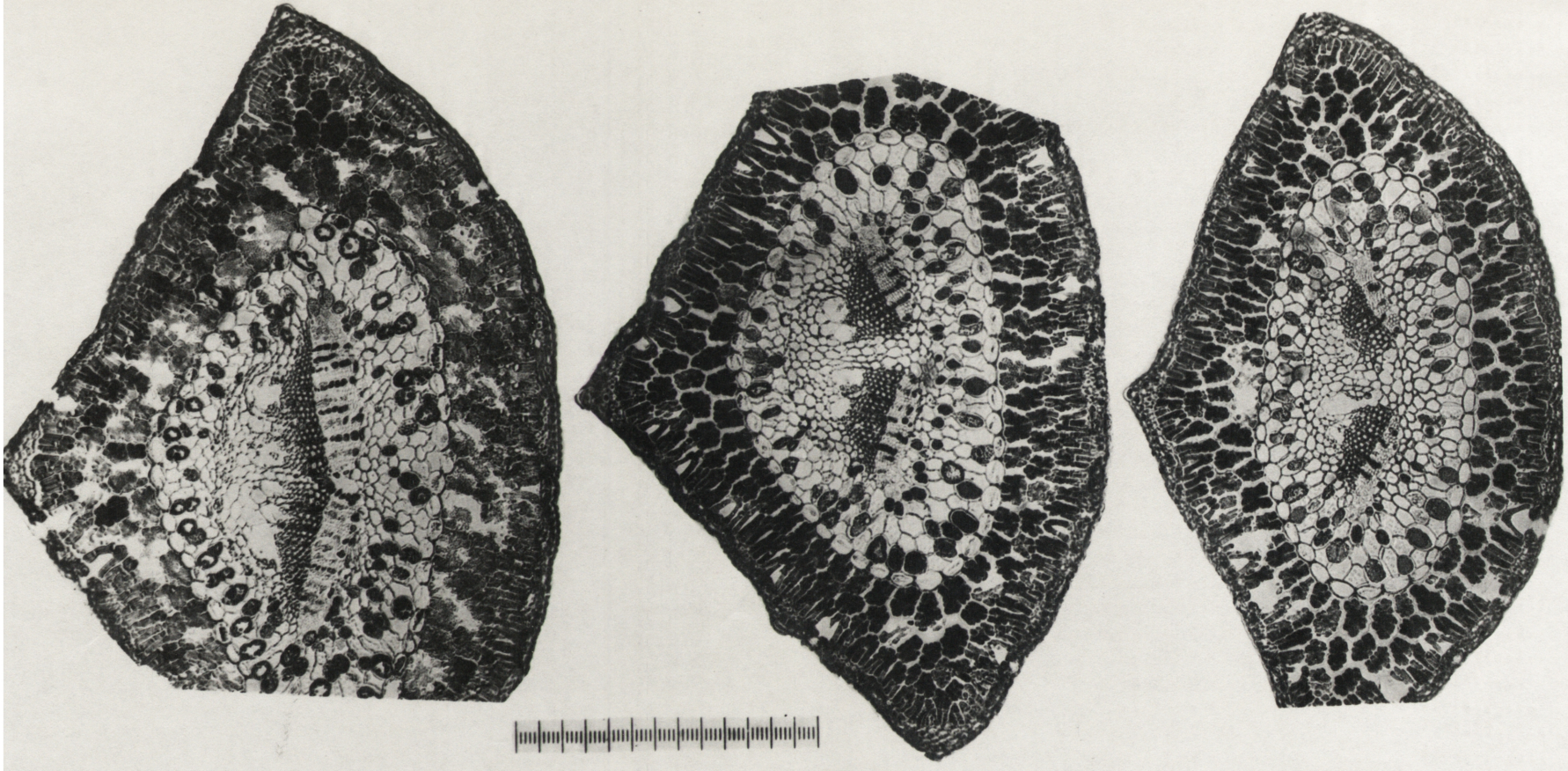
Upper section : 1-year-old foliage 15' above ground

Middle section : 2-year-old foliage 9½' above ground

Lower section : 3-year-old foliage 6' above ground

Sections were photographed under low power at a magnification of 50X.

Each minor scale division represents 0.01 mm.



(Plates 7 and 8). The lesser staining of this tissue may be indicative of reduced chlorophyll levels (cf. Table 19).

DISCUSSION

It is essential to interpret the results of correlation analyses with caution because it is possible to obtain highly significant correlations between factors which biologically have no relation whatsoever. It has been demonstrated repeatedly, however, that the size and development of conifer needles are affected both by chronological age and by the height of their insertion in the canopy (Helmers, 1943; Freeland, 1952; Nyestsyarovich et al., 1963; Hall, 1966), which supports the findings of this study (Tables 16 and 17). It is suggested that the height association is probably causal in so far as height might be expected to integrate the effects on leaf growth and development of the internal distribution patterns of moisture, nutrients, and hormones, and of the gradients of factors in the aerial environment.

The evidence of an increase in needle size with age at a given level in the crown (Plate 7; and compare, for example, the data for samples 1, 2, 7, and 10 - Table 16) suggests at first sight that the basal meristem of a needle may remain active for a number of years. However, some or all of the size advantage of the older needles may be due to the better light conditions under which they developed. The 4-year-old needles (Table 16), for example, developed on the tree when it was two years old and when light conditions would have been

optimum for needle growth. In contrast, the 1-year-old foliage at the same level in the crown developed when the tree was five years old. By this time, the intensity of light at the particular level in the crown (2'-3' from ground) would have been low (Appendix 10, Figure A3). The relatively small differences in the size and structure of 1-, 2-, and 3-year-old stem needles (Plate 8) are not unexpected as each set of foliage developed under full sun conditions, and, possibly, under optimal internal conditions (each set developing on the apical shoot).

As a needle ages chronologically and grows, correlated changes occur in the length of the fascicle sheath and in the number of stomatal rows per needle (Table 17). The variation in the former parameter with height of insertion in the canopy (as well as with age) implies that the length of the fascicle sheath is useless as a diagnostic feature for identifying P. radiata. In some other species of Pinus, the length of the sheath presumably remains reasonably constant throughout the crown as it is used as an aid in species identification (de Vall, 1940). The evidence that the number of stomatal rows on the abaxial face of a needle, sampled from any crown position, is linearly related to needle thickness (Figure 27) is consistent with an earlier finding on seedlings in the laboratory that the number of rows per mm of needle thickness is unaffected by the light climate under which seedlings are grown (Appendix 5, Table A11). The relationship warrants close examination in the genus Pinus. If it applies generally, it may

provide a basis for studying the adaptive significance of variation in stomatal frequency in Pinus species, a subject about which little is known (see Thames, 1963; Squillance, 1966).

No physiological significance can be attached to the poorly defined trend of a decrease in the moisture content of needles with age (Table 16). Needle moisture content is an unreliable measure of leaf water stress as it may be altered by dry weight changes without any change occurring in leaf water content (Clausen and Kozlowski, 1965a, b).

The differences in size and structure between needles sampled from the upper and lower crown (Plate 6) suggest that, even in a 6-year-old plantation, the foliage has differentiated into 'sun' and 'shade' types. For example, the thicker leaves in the upper crown with their better developed vascular system and palisade and sponge mesophyll tissue are features which are characteristic of 'sun' foliage (Appendix 6). While the differences may be due partly to the light gradient in the forest, ontogenetic effects and differences in physiological conditions within the tree crown could have substantially influenced leaf size and development (Kramer and Kozlowski, 1960; Leopold, 1964).

The physiological significance of the anatomical differences observed in foliage sampled from different positions in the tree crown is impossible to assess at this stage. More detailed studies of leaf structure and measurements of leaf diffusive resistances are

necessary for such an evaluation. However, the differences in the physical size of needles and in the amount of chlorenchyma they contain are so marked that one might expect to find differences in the photosynthetic capacity of foliage.

It is apparent that while the basic structure of the needle, which is under genetic control, is unaltered, external environmental factors and internal physiological conditions control the physical size of the organ at different positions within the tree crown.

STUDY 7

SPATIAL VARIATION IN LEAF CHLOROPHYLL WITHIN THE CROWN OF A PLANTATION SAPLING

Because of the importance of chlorophyll to photosynthesis, the total chlorophyll content of a vegetated area is part of the basic data of primary productivity studies (Jahnke and Lawrence, 1965; Lieth, 1965). Despite this, the volume of published data on the chlorophyll content of coniferous species is surprisingly small, and what data are available are difficult to collate because many methods have been used to express concentration, viz., fresh weight, dry weight, surface area, volume, concentration per needle, etc. No single method of expression is entirely satisfactory, but surface area is usually regarded as being most suitable as the absorption of radiant energy is determined largely by the surface of the plant organ presented to the light source.

The aim of the study was to assess the spatial distribution of chlorophyll within the crown of a young plantation tree of P. radiata.

METHODS

Field procedure: The sample tree and sampling positions were identical to those used in the previous study. 4-8 ternate fascicles (the number depending on needle size) were detached from each sampling position and placed in sealed, labelled, plastic bags. Sampling

was first conducted on 9/11/65 and repeated on 20/1/67. The latter sampling was undertaken to check whether the spatial distribution of chlorophyll evident in the results of the November 1965 sampling (period of severe drought) was modified in a more favourable season. Sampling in both years was undertaken between 0900-1100 hours to avoid any effects of diurnal variation in chlorophyll concentration (see Jayarmireddy and Rao, 1965). The procedures used in foliage preparation, pigment extraction, and spectrophotometric analysis are described in Appendix 3. In this instance the leaf chlorophylls alone were examined. Concentrations have been expressed on a fresh weight, dry weight, surface area, and volume basis to facilitate comparison with other data. The surface area and volume of the extracted sample material were determined from the length of the extracted sections (5 cm) and their mean 'radius' using the principles and formulae described in Appendix 1.

Computation: As the plotted data (pigment concentration by needle age class with height in the canopy) appeared to fit approximately linear or quadratic functions, they were analysed by multiple regression using the model described in the previous study.

RESULTS

Full results from the November 1965 and January 1967 samplings are presented in Appendix 8, Tables A39-A42.

Chlorophyll-a, chlorophyll-b, total chlorophyll, and the Ca/Cb ratio are all highly correlated with needle age and/or the height of needle insertion in the canopy (Table 18), the relative importance of the age and height terms in the regression depending on the basis used to express chlorophyll concentration, e.g., with surface area, the height term is more important, whereas with dry weight, the age term is more important.

Patterns evident in the data may be summarised as follows:

(i) Spatial variation in the concentrations of Ca, Cb, and Ca+b in needles alters depending on the basis used to express pigment concentration. Concentrations based on surface area and volume give the most divergent patterns. Those based on fresh and dry weight are intermediate (Figures 29-32).

(ii) The concentrations of Ca, Cb, and total chlorophyll, when expressed on a surface area basis, are largely unaffected by needle age but increase 2-3 fold from the base to the top of the crown. In contrast, when concentration is expressed on any of the other bases, it decreases with age, the change in concentration from the base to the top of the crown rarely exceeding $\pm$ 30 per cent, and being more usually within $\pm$ 10 per cent, of that at the crown base (Figures 29-32).

(iii) Pigment concentration in the needle reaches a maximum in the first year (though the trends alter somewhat depending on the basis used to express concentration - Figure 33). Whether or not the maximum level persists for an additional year may depend on

TABLE 18 Results of the multiple regression analyses using chlorophyll content 9/11/65 as the dependent variable (Y), and needle age in years (B) and height of insertion in the canopy in feet (C) as the independent variables

Dependent variable	Regression equation Δ	Correlation coefficient (r)	Significance of regression ϕ
Chlorophyll-a (mg/dm^2)	$Y = -0.248 + 0.554B - 0.068B^2 + 0.364C - 0.012C^2 - 0.057BC$	0.98	***
" (mg/cm^3)	$Y = 1.552 - 0.534B + 0.069B^2 - 0.018C - 0.001C^2 + 0.018BC$	0.96	***
" (mg/g dry wt.)	$Y = 2.572 - 0.375B + 0.046B^2 + 0.066C - 0.002C^2 - 0.023BC$	0.97	***
Chlorophyll-b (mg/dm^2)	$Y = 0.129 + 0.163B - 0.018B^2 + 0.095C - 0.002C^2 + 0.015BC$	0.96	***
" (mg/cm^3)	$Y = 0.812 - 0.267B + 0.034B^2 - 0.045C + 0.001C^2 + 0.013BC$	0.90	***
" (mg/g dry wt.)	$Y = 1.447 - 0.240B + 0.030B^2 - 0.062C + 0.004C^2 + 0.002BC$	0.83	**
Chlorophyll-a+b (mg/dm^2)	$Y = -0.109 + 0.725B - 0.088B^2 + 0.456C - 0.015C^2 - 0.072BC$	0.98	***
" (mg/cm^3)	$Y = 2.364 - 0.802B + 0.103B^2 - 0.064C + 0.001C^2 + 0.031BC$	0.95	***
" (mg/g dry wt.)	$Y = 4.020 - 0.616B + 0.076B^2 + 0.004C + 0.002C^2 - 0.022BC$	0.95	***
Ca/Cb	$Y = 1.575 + 0.159B - 0.025B^2 + 0.023C - 0.010C^2 - 0.023BC$	0.95	***

ϕ ** indicates significance at $p < .01$

*** indicates significance at $p < .001$

Δ Some terms in the equations presented were non-significant based on the t-test, but they are not excluded because, with more extensive sampling, they could attain significance (based on advice of G.A. McIntyre, Division of Mathematical Statistics, C.S.I.R.O.). The chlorophyll data based on surface area, however, may well fit a model based on the height terms alone.

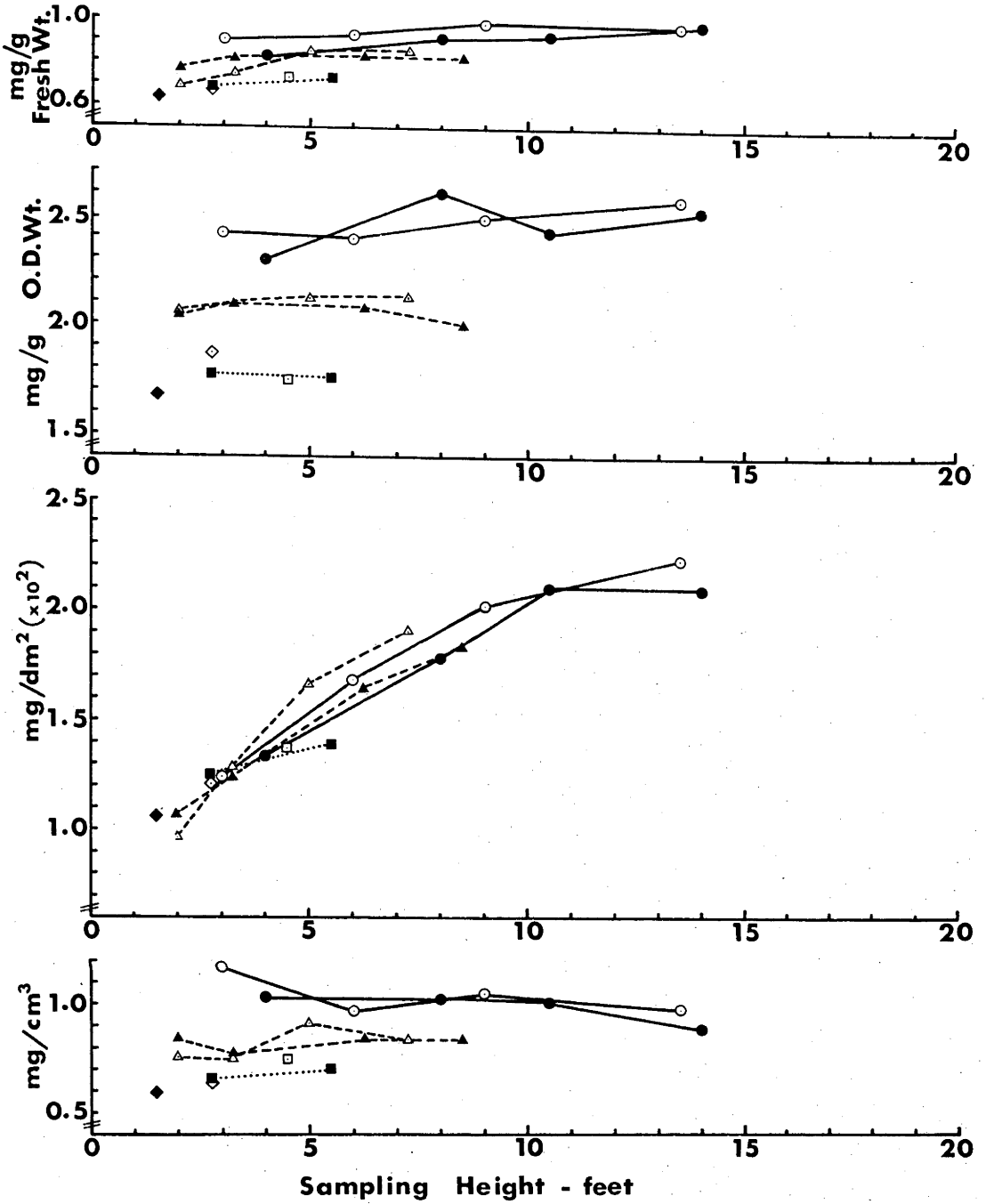


FIGURE 29 : Spatial variation in the concentration of chlorophyll-a in the crown of a 6-year-old P. radiata plantation tree 17½ feet tall. Sampled in November 1965 during a severe drought.

} 1-year foliage
} 2- " "
} 3- " "
} 4- " "

Open and closed symbols indicate sampling from N and S sides of tree respectively.

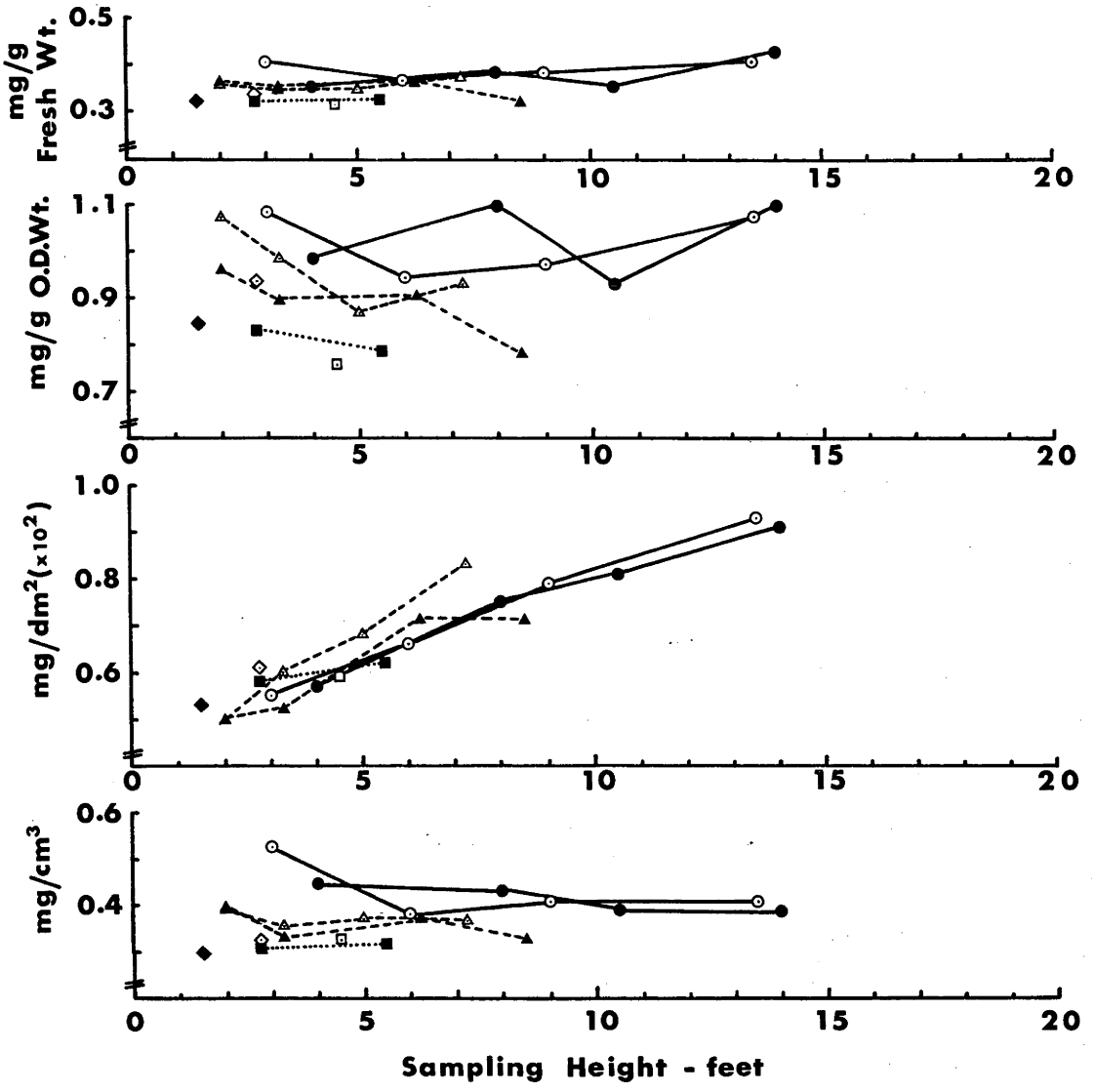


FIGURE 30 : Spatial variation in the concentration of chlorophyll - b in the crown of a 6 - year - old P. radiata plantation tree 17½ feet tall. Sampled in November 1965 during a severe drought.

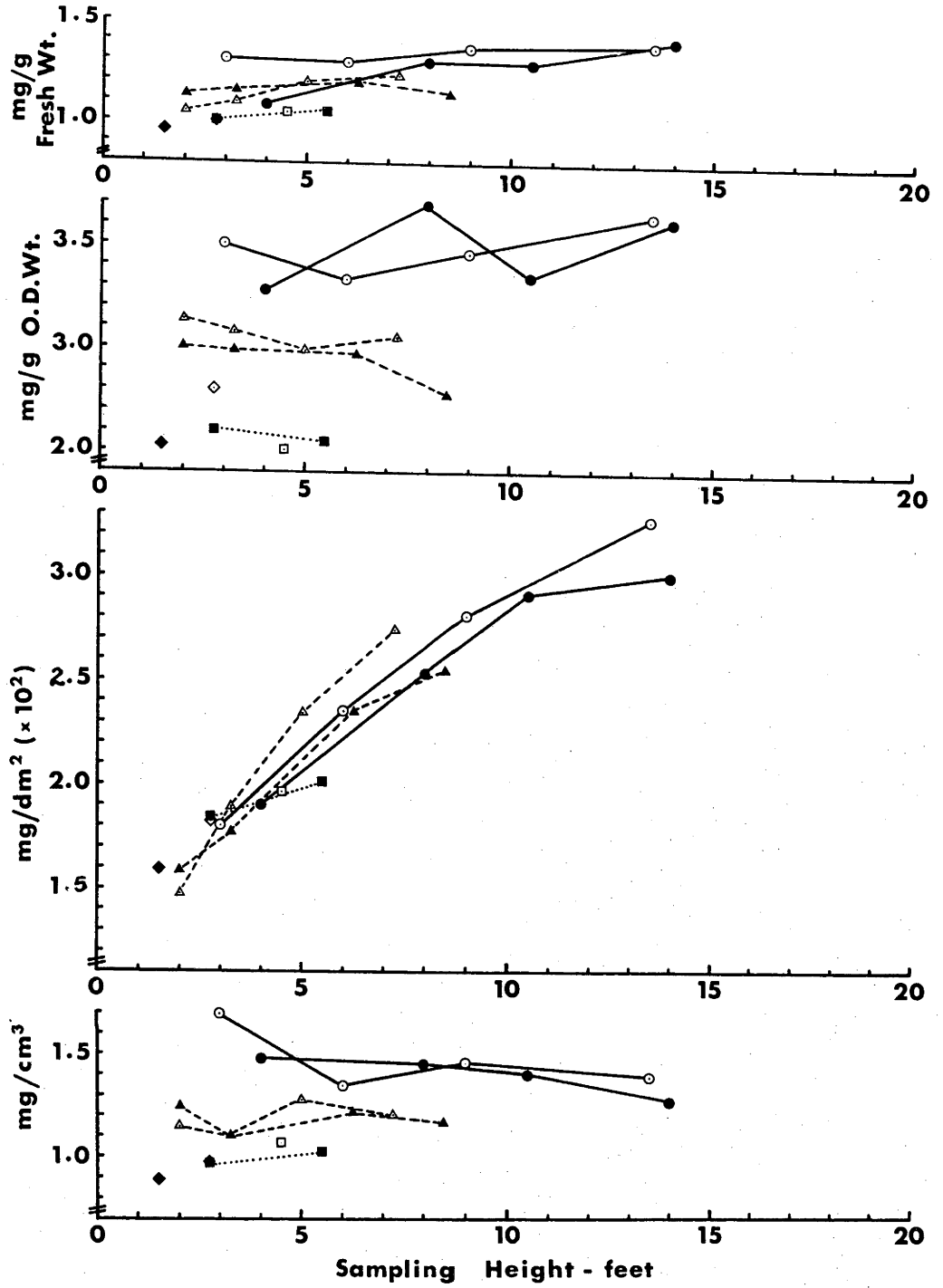
} 1 - year foliage

} 2 - " "

} 3 - " "

} 4 - " "

Open and closed symbols indicate sampling from N and S sides of tree respectively.



Corrected S/ls 7/6/71

FIGURE 31 : Spatial variation in the concentration of total chlorophyll (a+b) in the crown of a 6-year-old P. radiata plantation tree 17½ feet tall. Sampled in November 1965 during a severe drought.

} 1-year foliage
} 2- " "
} 3- " "
} 4- " "

Open and closed symbols indicate sampling from N and S sides of tree respectively.

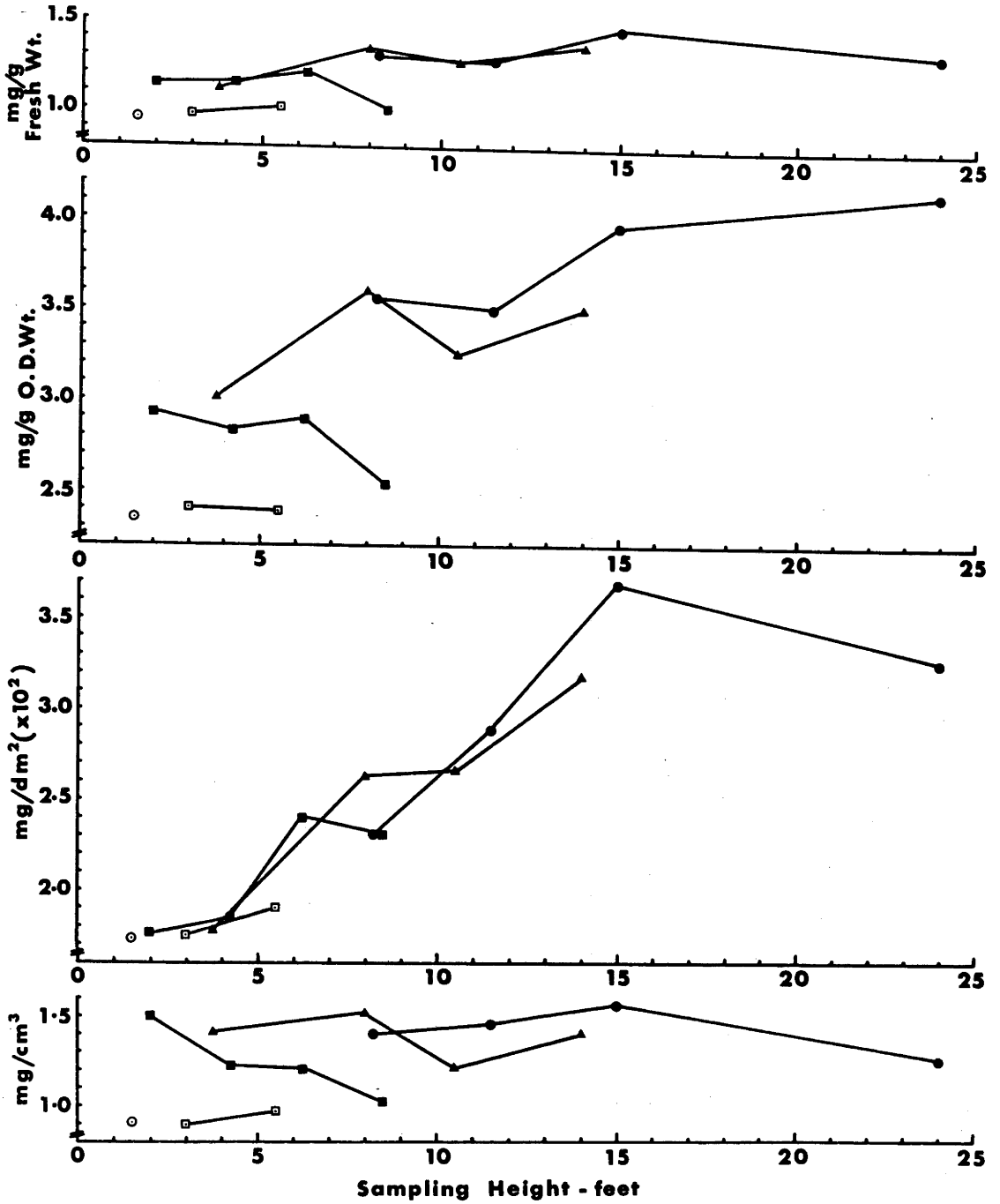


FIGURE 32: Spatial variation in the concentration of total chlorophyll (a+b) in the crown of a 7½-year-old P. radiata plantation tree 29 feet tall. Sampled in January 1967 following a good season.

- 1-year-old foliage
- 2- " - " "
- 3- " - " "
- 4- " - " "
- 5- " - " "

Sampling confined to N side of tree.

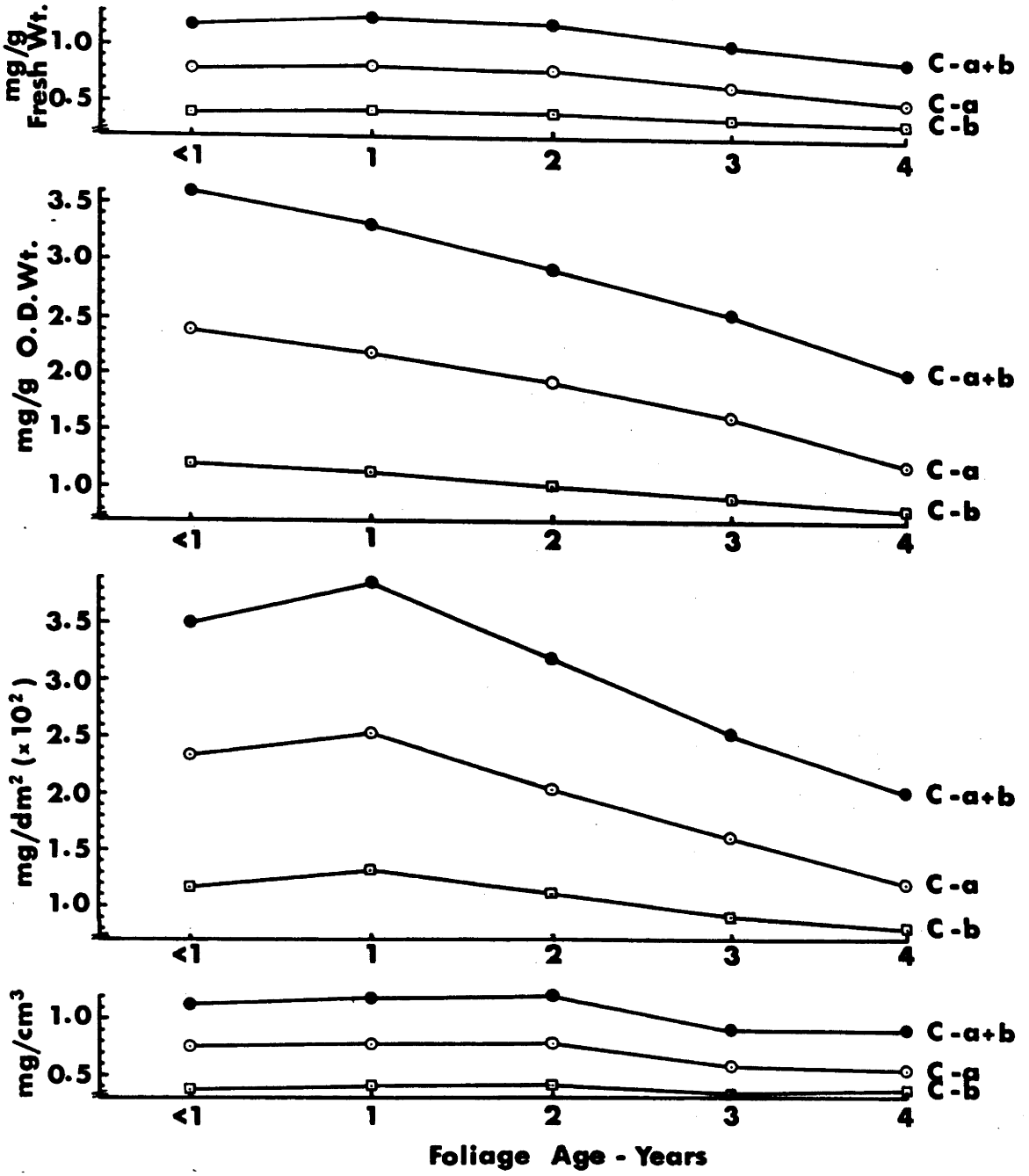


FIGURE 33 : Effect of foliage age on the concentration of chlorophyll-a, chlorophyll-b, and total chlorophyll in stem fascicles of a 7½-year-old P. radiata plantation tree 29 feet tall. Sampled in January 1967 following a good season.

seasonal conditions, e.g., in 1965, a drought year, the pigment concentration was highest in 1-year-old foliage (Figures 29-31). In 1967, on the other hand, following a good season, the concentrations in 1- and 2-year-old foliage were comparable (Figure 32).

(iv) A change in seasonal conditions does not affect the basic patterns of variation in pigment concentration with height of needle insertion in the canopy, e.g., compare Figures 31 and 32.

(v) The Ca/Cb ratio of foliage within the canopy increases from ground level to mid-crown height and then levels off (Figure 34).

A comparison of the concentrations of pigments in that foliage which was sampled both in November 1965 and January 1967 (Table 19) reveals an interesting phenomenon. Irrespective of needle age, the total concentration of chlorophyll in each sample barely altered over the 14-month period; however, the concentration of chlorophyll-a generally decreased while that of chlorophyll-b invariably increased. As a result, there was a marked fall in the Ca/Cb ratio.

DISCUSSION

Results indicate that the effects of needle age on the concentration of pigment in the crown are prominent when concentration is expressed on a weight or volume basis, but not on an area basis; whereas the effects of height of insertion in the crown are the reverse, i.e., they are prominent only on an area basis (Figures 29-32). The most likely explanation is

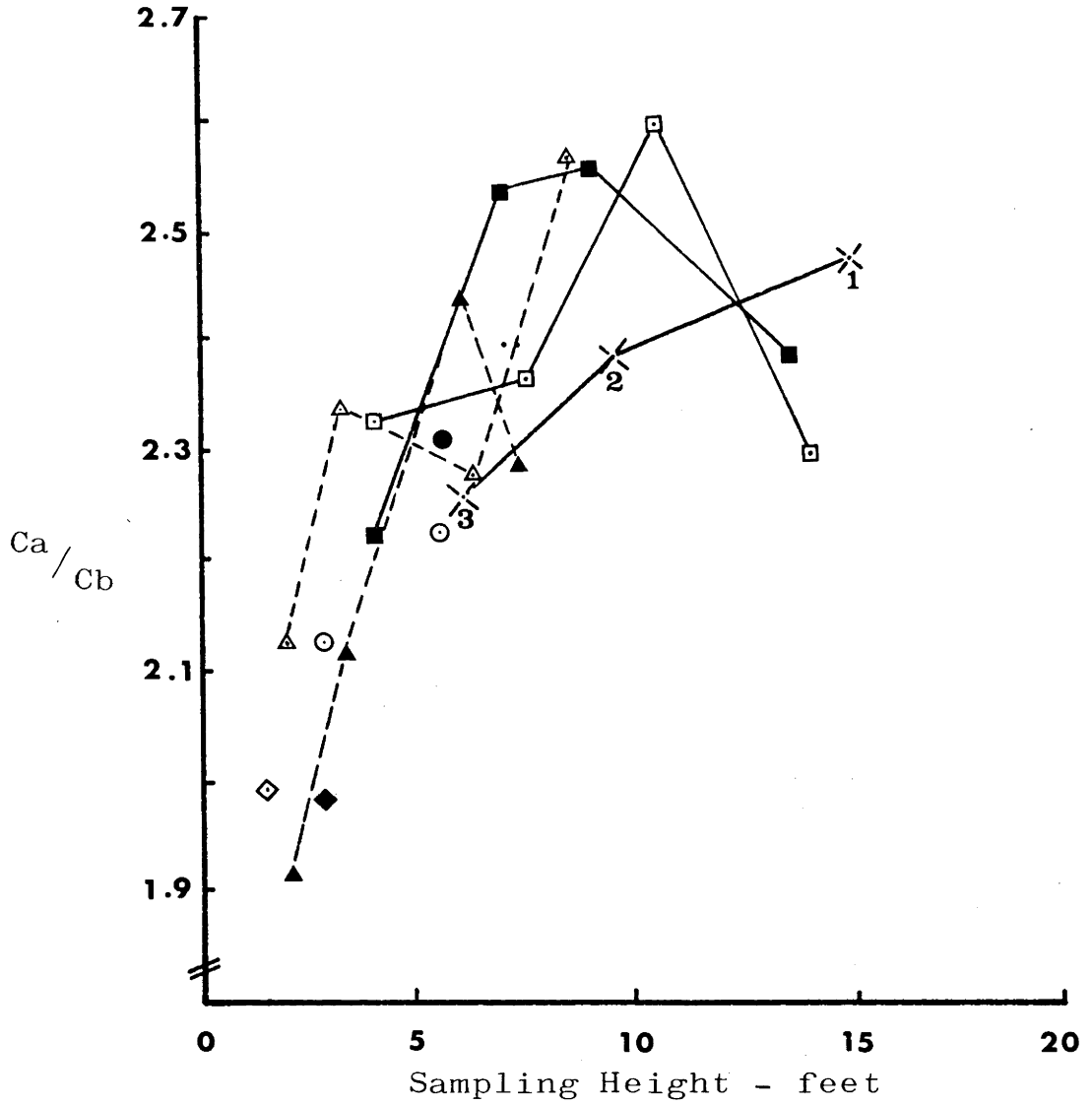


FIGURE **34 :** Spatial variation in the ratio of chlorophyll-a to chlorophyll-b in the crown of a 6-year-old P. radiata plantation tree 17½ feet tall. Sampled in November 1965 during a severe drought.

- 1-year foliage on N side of tree
- 1-year " " S " " "
- 2-year " " N " " "
- 2-year " " S " " "
- 3-year " " N " " "
- 3-year " " S " " "
- 4-year " " N " " "
- 4-year " " S " " "
- Stem fascicles aged 3, 2, and 1 year(s) respectively.

TABLE 19: Variation in the chlorophyll content (mg/dm^2) of foliage sampled from identical positions in the crown of a 6- to 7-year-old P. radiata plantation tree on 9/11/65 (severe drought) and 20/1/67 (following good seasonal conditions)

Sample no.	Needle age (yrs)		Height (ft)	Chlorophyll-a		Chlorophyll-b		Chlorophyll-a+b		Ca/Cb	
	9/11/65	20/1/67		1965	1967	1965	1967	1965	1967	1965	1967
5	1	2	4	1.33	1.13	0.57	0.65	1.90	1.78	2.33	1.73
11	1	2	8	1.78	1.67	0.75	0.96	2.53	2.63	2.37	1.74
17	1	2	10⅝	2.10	1.74	0.81	0.92	2.91	2.66	2.60	1.90
21	1	2	14	2.09	2.04	0.91	1.14	3.00	3.18	2.30	1.78
2	2	3	2	1.07	1.09	0.50	0.67	1.58	1.76	2.13	1.63
6	2	3	3	1.24	1.19	0.52	0.66	1.77	1.85	2.34	1.79
12	2	3	6	1.64	1.47	0.71	0.93	2.35	2.40	2.28	1.58
18	2	3	8½	1.83	1.44	0.71	0.87	2.54	2.31	2.57	1.66
7	3	4	3	1.25	1.05	0.58	0.70	1.84	1.75	2.13	1.51
13	3	4	5½	1.39	1.16	0.62	0.75	2.01	1.90	2.23	1.55
3	4	5	1½	1.06	1.05	0.53	0.67	1.59	1.73	2.00	1.57
23*	1	2	15	2.45	2.09	0.99	1.11	3.44	3.20	2.48	1.89
24*	2	3	9½	2.08	1.62	0.87	0.91	2.96	2.54	2.39	1.78
25*	3	4	6	1.39	1.20	0.61	0.81	2.00	2.01	2.26	1.48

* Stem fascicles

that the relationship between needle surface area and weight (or volume) alters both with needle age and with height in the crown, e.g., for a given height, the surface area per unit dry weight of needle decreases with age and, for a given age, decreases with height (Figure 35). It may be a chance result that pigment concentration on an area basis appears to be largely independent of needle age; however, the chlorenchyma in a pine needle is distributed around the outer shell of the leaf, and it is not improbable that pigment concentration may be a simple function of needle surface area.

The total chlorophyll levels of 3.3-4.2 mg/g dry weight and Ca/Cb ratios of 1.7-2.6 observed in the field in 1-year-old 'sun' foliage (Tables A39-A42, Appendix 8) are comparable with the figures for seedlings raised under full sun conditions in the laboratory (Tables A23 and A25, Appendix 5). The data, however, differ from figures quoted for 'sun' foliage of P. radiata trees aged from 1-20 years in New Zealand, viz., 2.4-2.5 mg/g total chlorophyll and Ca/Cb ratios of 3.4 at age 1 year to 2.6 at age 20 years (Cameron, 1966). The reasons for the differences are not known.

It is significant that low Ca/Cb levels occur in foliage in the lower crown where light levels are low (Figure 34; cf. Vakula, 1962 and Tieszen and Bonde, 1967). As postulated earlier, the relative changes in the concentration of the two chlorophylls may represent chromatic adjustments by the plant enabling the foliage to be more efficient at light utilisation under the prevailing light conditions. Chlorophyll-b, for

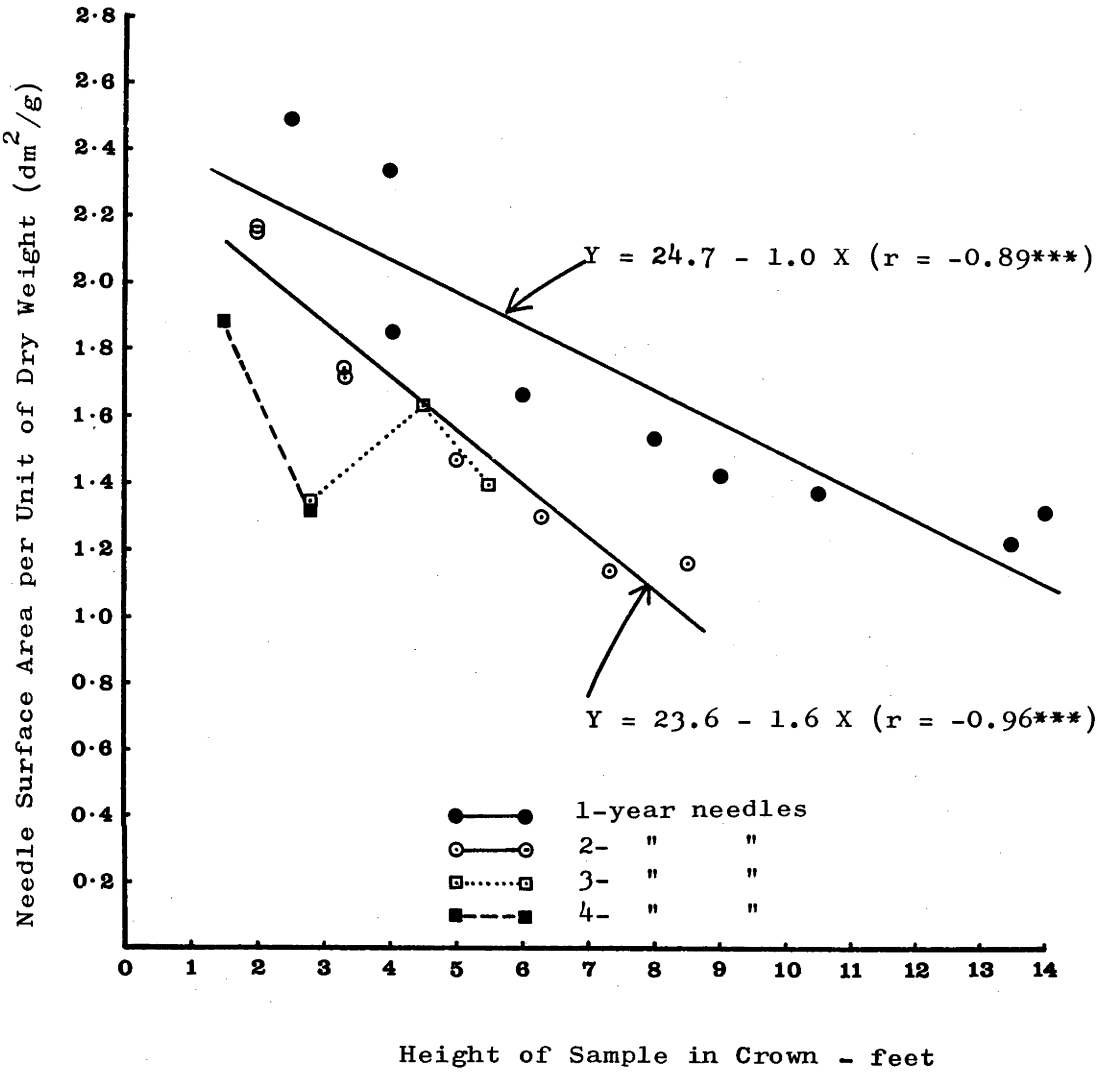


FIGURE 35: Variation in the surface area of needles per unit of needle dry weight in the crown of a 6-year-old P. radiata plantation tree

example, can improve the utilisation of light between 450-480 mμ. Light of these wavelengths is comparatively abundant in the blue-green shade of tree canopies (Rabinowitch, 1945).

While the effect of needle age on chlorophyll concentration in the P. radiata tree sampled varied somewhat with the basis used to express concentration (Figure 33), maximum levels occurred in foliage up to two years of age. Similar results have been reported for a number of evergreen tree species (Hiramatu, 1939; Reuther, 1948; Wood, 1967). The evidence that pigment levels were comparable in 1- and 2-year-old foliage in 1967, a good season (Figure 32), but higher in 1-year-old foliage in 1965, a drought year (Figure 31), suggests that seasonal conditions may affect the age at which pigment levels are at a maximum in needles. Seasonal conditions also appear to affect the total accumulation of chlorophyll in the leaf as the concentration of pigment in any one foliar sample in January 1967 was comparable with that measured on foliage sampled from the same position in November 1965, even for 3- and 4-year-old foliage (Table 19). This is contrary to the well established fact that pigment concentration in older leaves decreases with age. The anomaly is attributed to the severe drought conditions which existed prior to and at the time of the 1965 assessment. It is suggested that the much better seasonal conditions of 1966 improved the synthesis and accumulation of total chlorophyll sufficiently to negate the effect of age (cf. Spoehr and Smith, 1936). The improvement was mainly due to chlorophyll-b, but why the absolute levels

of this accessory pigment in early 1967 should invariably exceed those of late 1965 even in old foliage is not known (current trials under glasshouse conditions indicate that the accumulation of Ca relative to Cb is differentially affected by drought - Bacon and Wood, unpublished data).

Shading of P. radiata seedlings in the laboratory markedly increased the concentration of pigment in foliage when expressed on a dry weight basis (Appendix 5, Table A23). As light intensity within the tree crown decreases from top to base, one might have expected an increasing concentration of chlorophyll in needles (mg/g) with depth in the canopy. Generally, however, negligible and even reverse effects were found (Figures 29-32). Ontogenetic age differences between the two sets of experimental material may be partly responsible. It is possible, too, that patterns of pigment distribution in the crown of a forest tree are dominated by internal physiological conditions, viz., distribution patterns of nutrients, water, and hormones, against which the effects of shading become unimportant.

Whether pigment concentration is expressed on a surface area, weight, or volume basis, and assuming that the concentration of chlorophyll in the leaf and leaf photosynthetic capacity are positively correlated (Table 6), the distribution patterns of chlorophyll within the crown are such as to suggest that in the lower crown where light levels are low (Appendix 10, Figure A3) and older needles are concentrated (Figure 24), the photosynthetic activity of foliage may be

low. In contrast, a high activity might be expected in needles in the upper crown where light is abundant and the foliage is young. The concentration of pigment in this foliage, irrespective of the basis of expression, will be high.

STUDY 8

SPATIAL VARIATION IN THE RELATIVE WATER CONTENT AND PHOTOSYNTHETIC ACTIVITY OF FOLIAGE IN THE CROWN OF A PLANTATION SAPLING

The vertical layering found in the canopy of a forest or agronomic crop, and the modification of atmospheric conditions and processes within it, pose questions about the physiology of the crop, not the least important of which is the contribution which foliage in the various layers makes to crop growth. Foresters have frequently endeavoured to assess this indirectly by testing the effect on tree growth of pruning various percentages of the green crown (e.g., Stein, 1955; Slabaugh, 1957; Möller, 1960; Fielding, 1964; Shepherd, 1961, 1967), but few investigators have tackled the problem on a physiological basis. The study described here was an attempt to assess the relative activity of foliage in various sections of the crown of a young P. radiata plantation tree following a consideration of the spatial variation in the crown of the RWC and photosynthetic capacity of foliage.

METHODS

First order branches facing north-west in each of the 1961-6 spring whorls of the sample tree were the source of material for the measurement of gas exchange and relative water content.

Relative water content: The spatial variation in the RWC of foliage in the crown of the study tree was determined in August 1967. Samples were collected at dawn starting from the top of the tree and working downwards. They were taken from branches immediately adjacent to those from which shoots were detached for the measurement of CO_2-exchange. The fascicles in each sample were trimmed at the site and placed immediately in tared, stoppered test tubes containing 1 ml of distilled water. The samples were then taken to the laboratory where the RWC was determined using the technique described in Appendix 4.

The RWC of foliage on the branch used for the measurement of CO_2-exchange was also determined at the time of gas analysis. It was felt that this determination would give a better estimate of the water status of foliage on the detached shoot.

CO2-exchange: Foliage of each age on the first order branches previously described was sampled for the determination of CO_2-exchange capacity. The sampling was spread over a period of two weeks because a maximum of only two samples per day could be handled in the gas analysis system.

Foliar shoots were detached from the tree by cutting the shoot axis under water at 0830 hours each day, using the technique described earlier. Each shoot carried two ages of foliage except the shoots detached from branches in the 1966 spring whorl which carried 1-year-old needles only. The shoot, with its stub under water, was transferred to the laboratory and

the section carrying the younger foliage was sealed in the assimilation chamber. This order was chosen as preliminary trials suggested that the RWC of young foliage on a shoot detached under water might improve more rapidly than that of older foliage (cf. Gates, 1955). After acclimatising the shoot to the study conditions for one hour, the rate of CO_2-exchange was recorded on a descending series of illuminations (3500, 2500, 1500, 500, and 0 ft.c.) at 25 $\pm$ 1°C under constant air flow and relative humidity conditions. The section of the shoot carrying the younger foliage was then severed and the remaining section was sealed in the assimilation chamber. After a 1-hour acclimatisation period, the CO_2-exchange of the older foliage was recorded as previously.

Computation: A preliminary plotting of the data indicated that the RWC of foliage on the standing tree was dependent on needle age and height of insertion in the canopy, and the CO_2-exchange capacity of foliage, in addition, was dependent on the RWC. As the relationships appeared to be of a quadratic nature, the data were subjected to multiple regression analysis of the form:

RWC: $$Y = a_1+a_2B+a_3B^2+a_4C+a_5C^2+a_6BC$$

CO_2-exchange: $$Y = a_1+a_2B+a_3B^2+a_4C+a_5C^2+a_6BC+a_7D$$
$$+a_8D^2+a_9BD+a_{10}CD+a_{11}BCD$$

where Y is the dependent variable
B is the needle age in years

C is the height of needle insertion in the crown in feet

D is the RWC of foliage

a_1 is the regression constant

and a_2-a_{11} are the regression coefficients.

RESULTS

Relative water content: Results are presented in Appendix 9, Table A43, and are illustrated in Figure 36. Analysis of the data indicates that the RWC of needles was highly correlated with needle age and the height of insertion in the canopy ($r = 0.95$), the regression being significant at $p < .001$. The regression equation was:

RWC (%) = 96.3 - 3.730 Age + 0.083 Age x Height

where age is expressed in years and height in feet.

In the material sampled, needle age was the dominant control over the RWC, the 't' test showing it to be significant at $p < .001$ and the Age x Height factor at $p < .05$. An increase in needle age lead to a marked reduction in the relative water content of needles.

CO2-exchange: The data from the CO_2-exchange study are presented in Appendix 9, Table A44, and are illustrated in Figure 37. The results of the correlation analyses based on them are presented in Table 20. The significance of regression in all cases except one was $p < .01$. The regression of the compensation point for

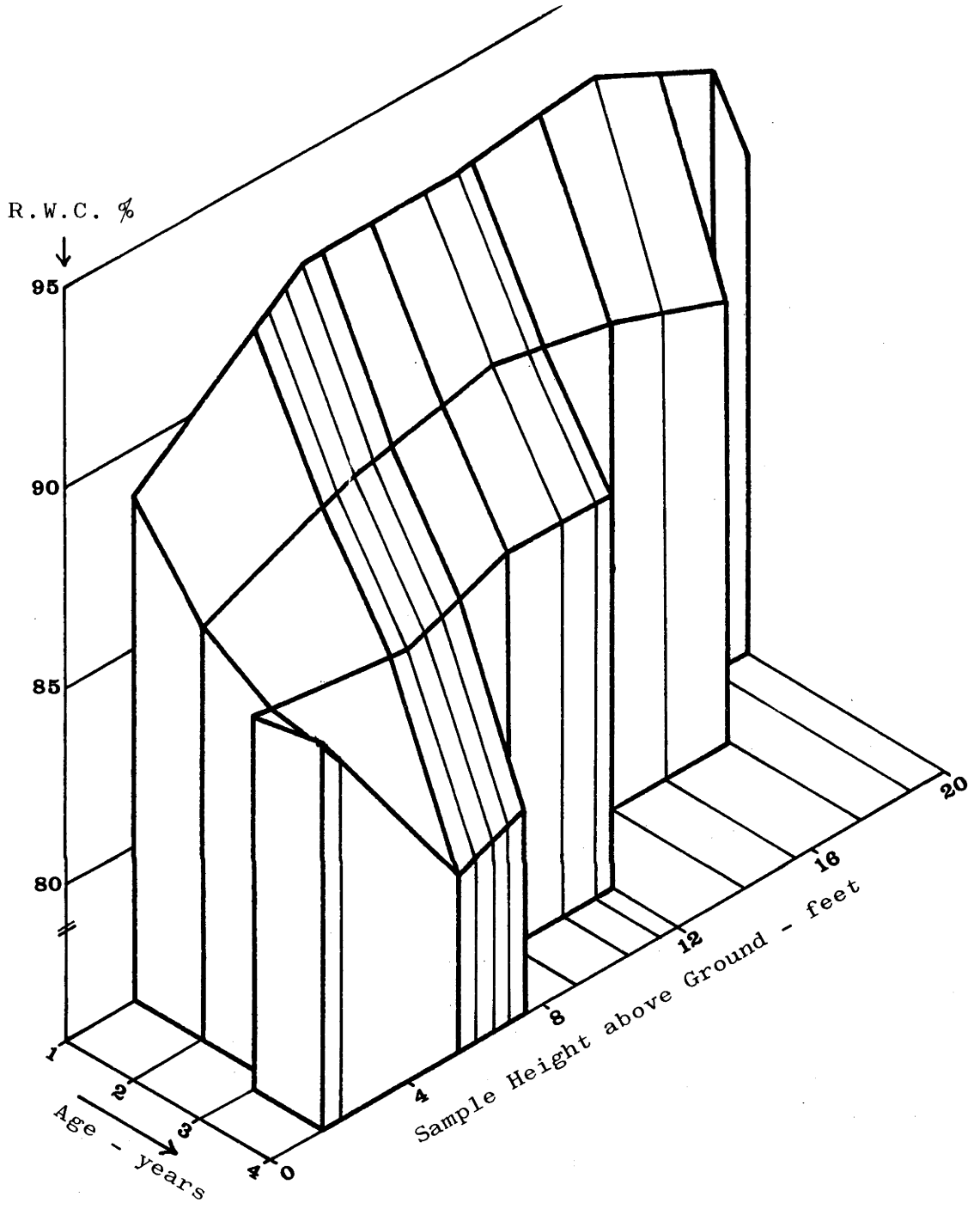


FIGURE **36** : Spatial variation in the relative water content (R.W.C.) of foliage in the crown of a 7-year-old P. radiata tree 24 feet tall.

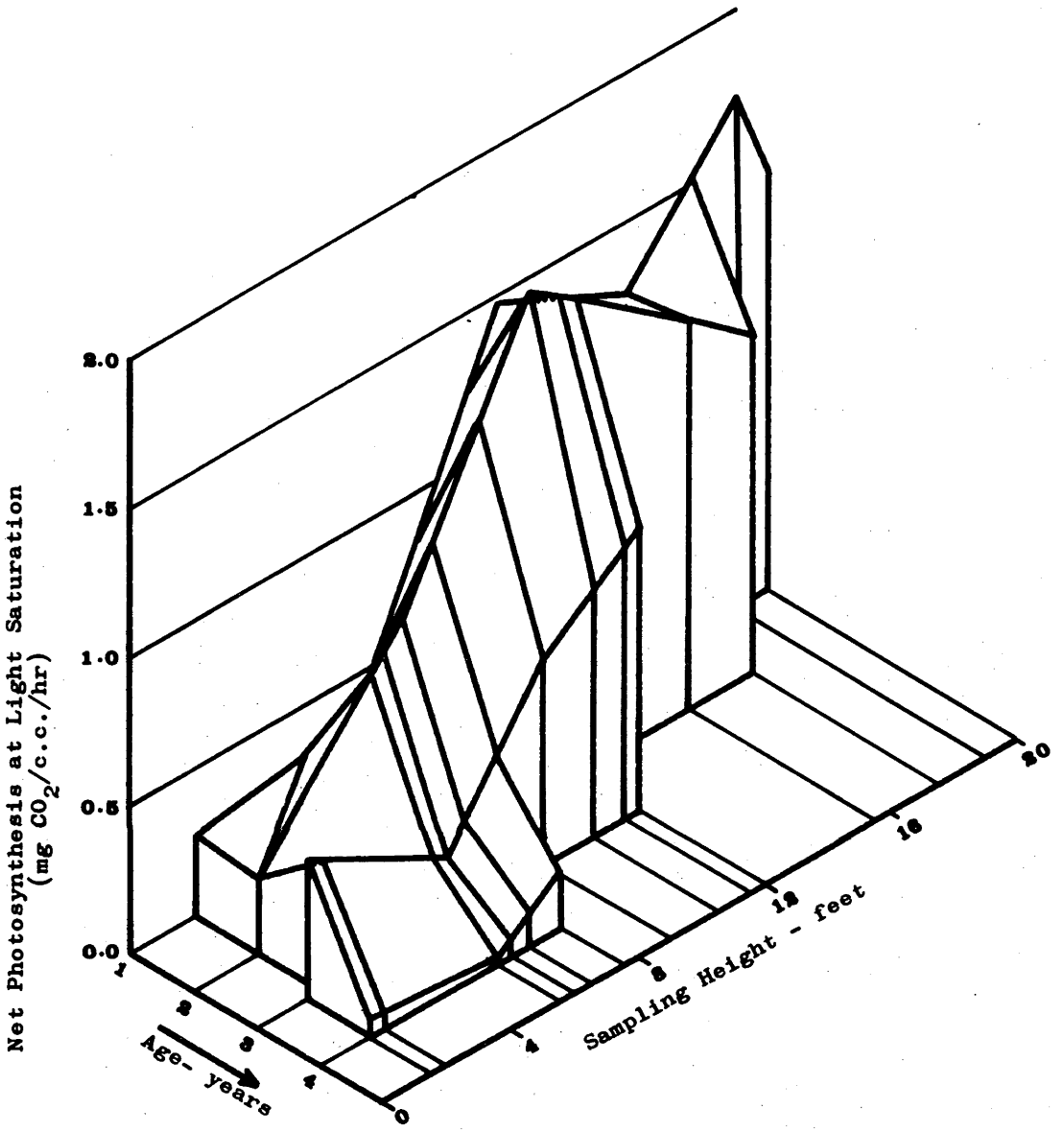


FIGURE 37 : Spatial variation in the rate of net photosynthesis at light saturation of foliage detached from the crown of a 7-year-old P. radiata tree 24 feet tall.

light was significant at $p < .05$, the lower level of significance being due largely to the exceptionally high compensation point (3800 ft.c.) of 4-year-old needles in the 1962 spring whorl. This abnormal value probably reflects the effects of both age and water stress. There is evidence of a low RWC in the 4-year-old foliage (Figure 36).

The general trends evident in the data are:

(i) The rate of net photosynthesis at light saturation was highest in 1- to 2-year-old foliage and increased with height in the canopy for all ages of foliage (Figure 37). The photosynthetic activity of foliage of various ages at a given height in the canopy was, in descending order, age 2 $\geqslant$ age 1 $>$ age 3 $\gg$ age 4.

(ii) The RWC of foliage was highly correlated with the rate of net photosynthesis at light saturation. A 't' test showed the RWC, RWC^2, and Height x RWC terms to be significant at $p < .01$, and the Age x Height term at $p < .05$ (Table 20). As light intensity was reduced, the age and height terms exerted a greater influence on the regression.

(iii) The rate of dark respiration increased with foliage age and with the height of needle insertion in the canopy (Figure 38). In addition, it was inversely correlated with the RWC of foliage (compare Figures 36 and 38), tending to increase with a decrease in the RWC. (Note: The age scale in Figure 36 is the reverse of that in Figure 38. This was necessary for clarity.)

TABLE 20 Results of the multiple regression analyses using various parameters in CO_2-exchange as the dependent variable (Y), and needle age in years (B), height of insertion in the canopy in feet (C), and relative water content in per cent (D) as the independent variables

Dependent variable	Regression equation	Correlation coefficient	Significance of regression ∮
Net P at light saturation (mg CO_2/c.c./hr)	$Y = -125.255 - 0.032\,BC - 0.005C^2 + 0.003CD + 2.866\,D - 0.016D^2$	0.93	***
Net P 3500 ft.c. (mg CO_2/c.c./hr)	$Y = -116.630 - 0.031BC - 0.006C^2 + 0.003CD + 2.663D - 0.015D^2$	0.93	***
Net P 2500 ft.c. (mg CO_2/c.c./hr)	$Y = -107.621 - 0.029BC - 0.006C^2 + 0.003CD + 2.454D - 0.014D^2$	0.91	***
Net P 1500 ft.c. (mg CO_2/c.c./hr)	$Y = -89.923 + 0.335B - 0.094C - 0.007C^2 + 0.012CD + 2.181D - 0.013D^2$	0.90	**
Net P 500 ft.c. (mg CO_2/c.c./hr)	$Y = 3.198 - 1.462B + 0.041C - 0.002C^2 - 0.033D + 0.017BD - 0.0002\,BDC$	0.90	**
Dark respiration (mg CO_2/c.c./hr)	$Y = 48.124 - 2.397B + 0.078BC - 1.035D + 0.006D^2 + 0.027\,BD - 0.0010\,BDC$	0.98	***
Compensation point for light (ft.c.)	$Y = 984.20 + 9249.40B - 111.82BD + 0.471BCD$	0.72	*

∮ * indicates significance at $p < .05$

** indicates significance at $p < .01$

*** indicates significance at $p < .001$

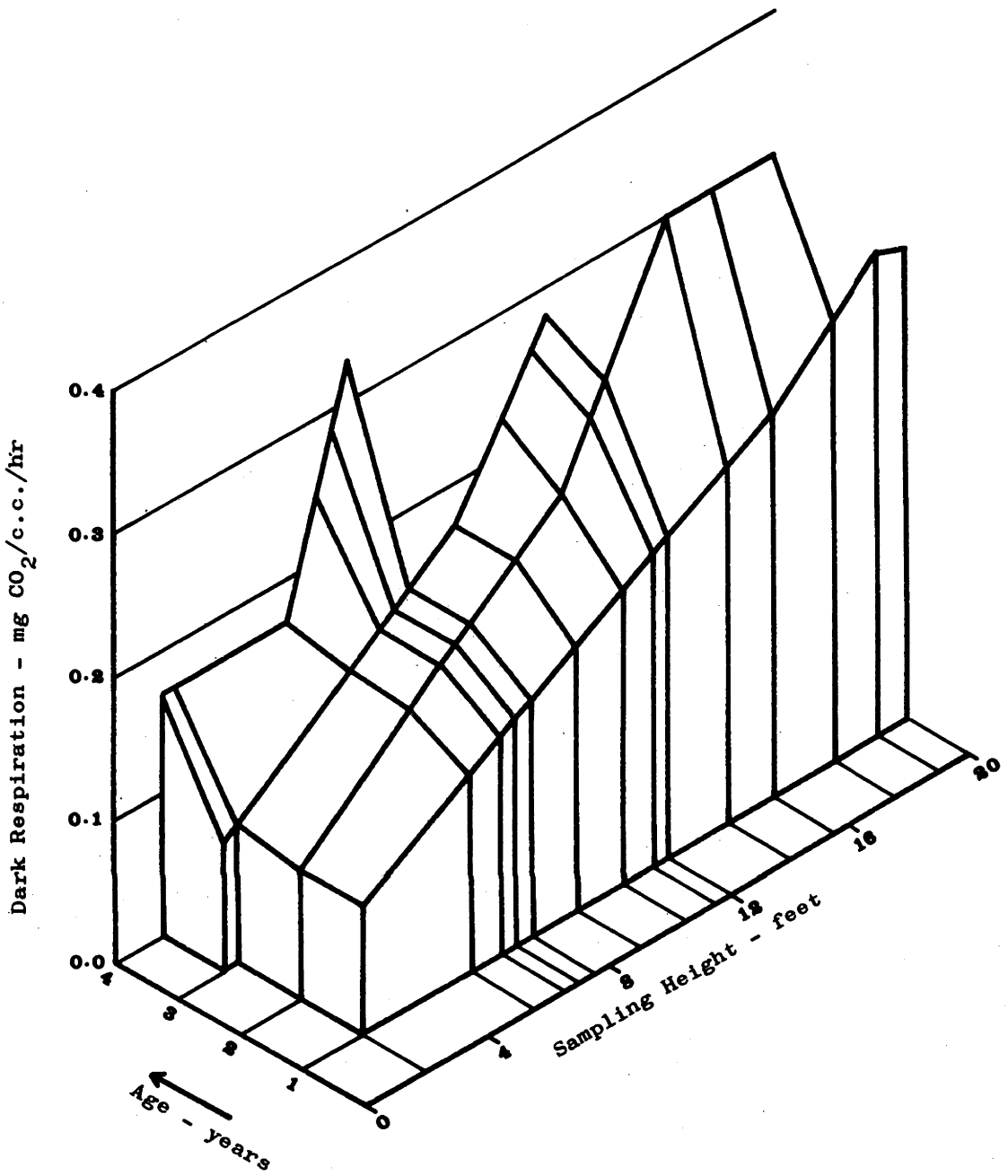


FIGURE 38 : Spatial variation in the rate of dark respiration of foliage detached from the crown of a 7-year-old P. radiata tree 24 feet tall.

(iv) The compensation point for light was strongly correlated with needle age (Figure 39) and to a lesser extent with the RWC of foliage and the height of needle insertion in the canopy. It was highest in the older foliage and in the upper levels of the canopy.

(v) The rates of net photosynthesis at light saturation were low or negligible in cases where the RWC of foliage was in the low eighties (Appendix 9, Table A44).

DISCUSSION

The significance to the physiology of the tree of the spatial variation observed in the RWC of foliage within the crown (Figure 36) is somewhat obscure without information on the relationship between the RWC and water potential of needles as they age. However, changes occurring within a leaf with age, e.g., increased dry weight, decreased cell wall elasticity, and decreased osmotic potentials, have been shown to result in a progressively lower leaf water potential for a given leaf water deficit (Knipling, 1967). Thus, as the RWC of needles in the tree studied decreased markedly with age (Figure 36), one might be justified in assuming that the older foliage was subject to more severe water stress than the younger foliage. This would partly explain (needle age effects per se would also be involved) both the high correlation demonstrated between needle RWC and photosynthetic activity and the pattern observed in the spatial variation within the crown of the photosynthetic activity of foliage (Figure 37). The results prompt one to suggest that the

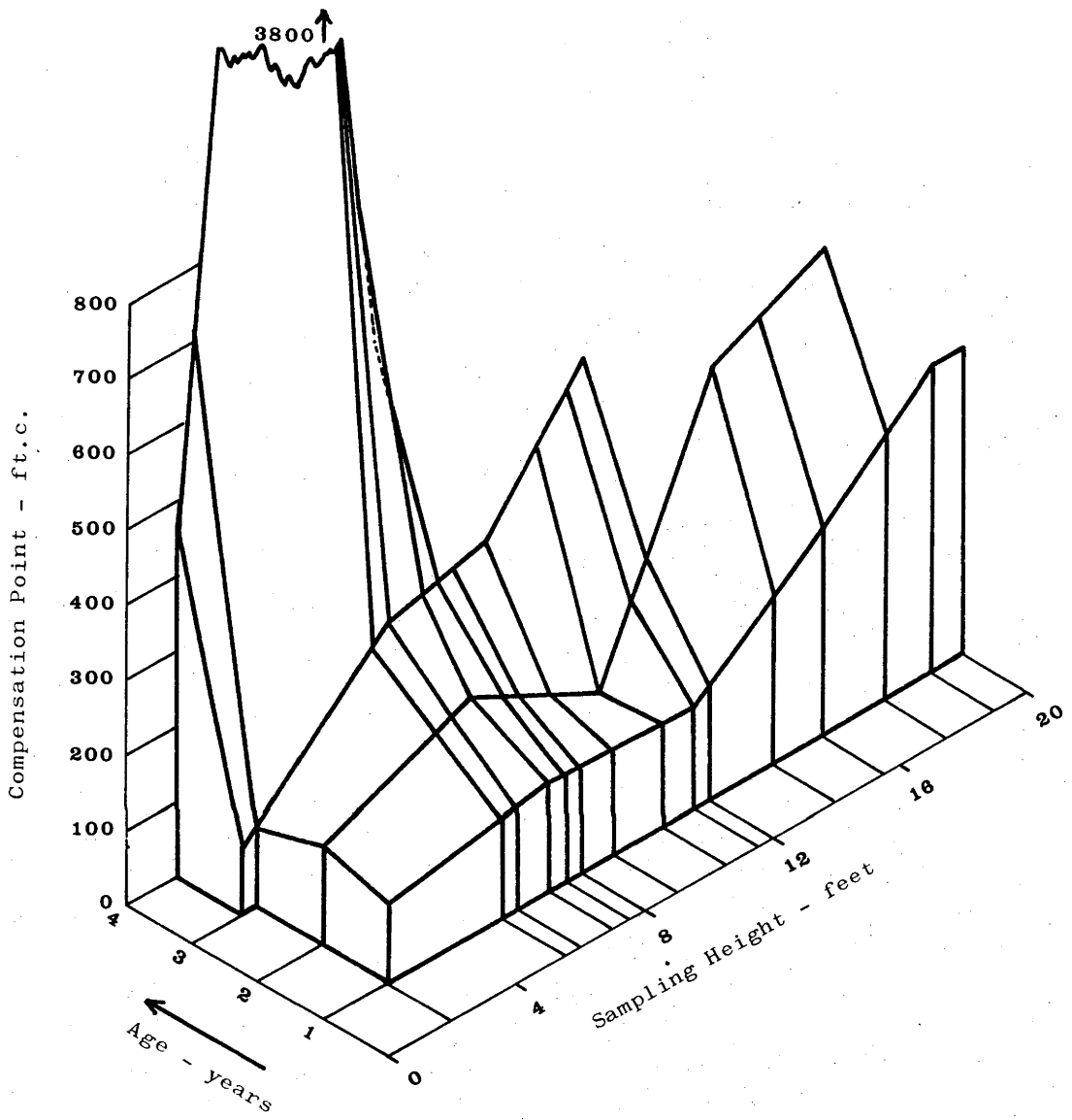


FIGURE **39** : Spatial variation in the compensation point for light of foliage detached from the crown of a 7-year-old P. radiata tree 24 feet tall.

distribution of moisture in the tree crown may be as significant as the distribution of light to the photosynthetic activity of foliage.

Changes in the capacities of all three partial processes in the photosynthetic system possibly have contributed to the variation evident in the photosynthetic activity of foliage within the crown (Figure 37). For example, the spatial variations observed earlier in the concentration of leaf chlorophyll and in leaf morphology could induce changes in the photochemical and diffusion processes respectively, while the spatial variation in the RWC of foliage implies differences in leaf water deficit which could affect all three partial processes (Slatyer, 1967). In addition, the metabolic activity of a leaf decreases with leaf age, and thus one might expect a change in the capacity of the biochemical process.

Maximum photosynthetic activity at a given height in the canopy occurred in 1- to 2-year-old foliage (Figure 37). This is consistent with the general finding in a wide range of plant species that photosynthetic activity builds up to a maximum at leaf maturity, and then declines (e.g., Koch and Keller, 1961; Hoffmann, 1962; Hodges, 1965). The close parallelism evident in the trends of pigment concentration and net photosynthesis with age (Figures 33 and 37) lends support to claims that changes in chloroplast activity may be partly responsible for changes in the photosynthetic activity of leaves as they age (Clendenning and Gorham, 1950; Thomas, 1965).

It was unexpected that the highest rates of dark respiration of needles, sampled from a given height in the canopy, would be found in older material (Figure 38). The young leaves of a wide range of plants (broadleaf types and conifers) almost invariably respire more rapidly than old leaves because of greater mitochondrial activity and the relatively higher proportion of protoplasm in relation to wall material in their cells (Geronimo and Beevers, 1964; Kozlowski and Keller, 1966). Leaf water stress, as indicated earlier, was probably severe in the older foliage (Figure 36) and may be the key to the phenomenon. However, the effects of water stress on the respiratory activity of leaves has not been satisfactorily resolved (see Kramer, 1959; Vaadia et al., 1961; Twersky et al., 1965).

Assuming that the magnitude of the dark respiration rate of foliage reflects the rate of photosynthesis preceding it (for which there is some evidence, e.g., Ludwig et al., 1965) the rates of shoots on the tree could vary continuously. Thus, the rates presented in Figure 38 may not be representative of the field situation.

Note: The discussion above is pertinent to samplings made on a single P. radiata plantation tree during its period of winter 'dormancy' and at a time of drought. Whether similar patterns occur in other trees of the species, and in other seasons and under more favourable environmental conditions, is a project for future research.

IV GENERAL DISCUSSION

The findings in this work cover several diverse aspects of photosynthesis and growth in Pinus radiata. Accordingly, such distantly related topics as the limitations of the data, the photosynthetic activity of foliage within the crown of a plantation sapling, and compensatory responses of seedlings to changes in environmental conditions are discussed.

The limitations of the data arise mainly from experimental variability and methodological errors. Whereas the sources of variation are known in some cases and appropriate corrections to a common standard are possible (e.g., RWC of foliage, p. 48), no satisfactory correction has been possible with the measurements of light intensity, temperature, and soil water. The main reason for this is the difficulty of specifically assessing each of these factors. Fortunately the errors involved in the measurement of the last two are known to be small. In contrast, the magnitude of the error involved in measurement of light intensity cannot be closely gauged. Errors of unknown magnitude are also involved in the methodological aspects when it is sought to relate the responses of severed shoots of seedlings to those of plantation trees (p. 68). Since the errors involved in the measurement of light intensity and temperature apply to almost every experimental observation, these are considered first.

Light intensity in all the photosynthetic work was measured inside the assimilation chamber at the same height as the top of the foliar shoot. Clearly, the energy recorded need not correspond with that absorbed by the leaf. The orientation of leaves with respect to the light source, their surface characteristics, and the effects of self-shading are some of the more obvious factors which may affect the amount of energy absorbed. The operation of such factors will lead to errors in the quantitative data relating the rate of photosynthesis of a leafy shoot to recorded light intensity. A particular shortcoming, however, is that photosynthetic gain in relation to unit of measured light must be expected to underestimate photosynthesis per unit of light absorbed by the leaf. Although the magnitude of these errors is not known, they are of the same type and direction in all observations. Thus, valid and useful comparisons can be made.

Similarly, comparisons should be valid in spite of the known but inassessable error in temperature measurement. Early attempts to monitor leaf temperature by inserting the junction of fine wire copper-constantan thermocouples in the leaf mesophyll tissue gave suspect readings. These suspect readings could have resulted from poor contact, stray voltages, or killing of tissue, and may have recorded essentially only air temperature. However, Pinus needles have a large transfer coefficient for sensible heat (Gates, 1965) and it is presumed, therefore, that leaf and air temperatures would not differ greatly. In the case of experiments with seedlings under fluorescent lighting

in a phytotron cabinet, since fluorescent lights have a low heating effect, it is expected that only small differences between leaf and air temperature would prevail. Thus, the results, though less precise than might be hoped for in the future, have real value.

The errors involved in estimation of the moisture content of soil in undrained pots containing seedlings (p.54 and Appendix 7) arise because the estimates are based on the assumption that the moisture content of roots relative to shoots is identical in seedlings raised in soil and in perlite. Although the amount of water in the plant shoot can be determined accurately, this does not apply to the estimation of the amount of water in the plant root system, the oven dry weight of which approximated 20g (Figure 16). However, as the dry weight of soil in each pot was 3184g, a gross error of about 30g in the estimate of root water would not affect the estimate of soil moisture content (%) by more than one unit.

Despite the errors of unknown direction and magnitude involved in equating the responses in severed shoots of seedlings to those from plantation trees, the rates of photosynthesis are of the same order in intact seedling shoots and detached tree shoots (0.4-2.6 and 0.0-1.7 mg CO_2/c.c./hr respectively). These comparatively small differences in photosynthesis may be associated with such factors as a probable high photosynthetic activity in primary leaves, differences in physiological and ontogenetic age, and other less evident endogenous factors. Most likely, the height in the tree crown from which the detached shoot was

obtained and response of the shoot to severance are further variables which are also involved. Even if the increase in the rate of photosynthesis (probably less than 20 per cent of initial - Figure 23) noted in seedlings in the several hours after severance of the shoot also occurs in cut shoots from trees, comparisons between shoots detached from different parts of a tree could be expected to remain unaffected. This is an important point when evaluating results relating to the variation of photosynthesis of foliage on shoots sampled from different parts of a tree crown.

On this basis, the evidence that needles within the crown vary in photosynthetic activity is clear and of interest. The finding that the most active foliage is on 1- and 2-year-old shoots, which were concentrated in the mid and upper levels of the canopy (Figure 24), is probably associated with needle age (cf. Freeland, 1952; Clark, 1961) as well as with the distribution of plant pigments and water within the crown. It is shown, for example, that pigment concentration was highest in young needles (Figures 29-33); that needle chlorophyll content and photosynthetic activity were positively correlated (Table 6); and that, at a given height in the canopy, the RWC of foliage at dawn was highest in young needles (Figure 36).

Together the 1- and 2-year-old needles accounted for approximately 80 per cent of the total weight of foliage in the crown of a 6-year-old plantation tree (Figure 25). Because of their position, a majority of these needles would be subject to light conditions optimum for photosynthesis (see Figure 24 and Appendix

10, Figure A3). As a result, most of the production of carbohydrate by the tree might be expected to result from leaf activity at the top and around the outer shell of the crown; but it was also found that the photosynthesis of foliage in the lower levels of the crown increased with increase in light intensity (Appendix 9, Table A44). This implies that production of carbohydrate by the tree might be significantly increased by thinning the surrounding forest.

The various facets of these field results were based on single tree studies at a single time. Different trends might be found in other individuals of the species at other times, not only because of phenotypic differences but also because the present study has demonstrated that environmental factors affect several physiological responses. In this regard, the environmental history is likely to be important. Nevertheless, the single tree studies show results of value, and this justifies further work.

Of particular importance is recognition that the most active photosynthetic region of the tree sampled was the zone of 1- and 2-year-old needles in the upper levels of the crown, where light was less often limiting, pigment concentration was high, and leaf water deficits were lower than elsewhere on the tree. If these relationships apply generally, then we have a physiological basis on which to examine critically the empirical schedules currently in use for pruning and thinning forest stands of this important commercial species.

There has been much discussion but no agreement as to whether the foliage on branches at the base of a tree crown is parasitic on the upper crown (cf. Bavngaard, 1957). In agronomic crops, some workers claim that the basal shaded foliage may be slightly parasitic on the upper crown (Burton and Jackson, 1964; Bleasdale, 1966) but this is disputed by others (Ludwig et al., 1965). Hopkinson (1966) even suggests that the basal foliage may serve as a source of mobile mineral elements. However, physiological comparisons of leafy agronomic crops with trees are not always valid, since the lower shaded leaves of an agronomic crop are not comparable with the lower branches of a tree which may bear foliage of several ages. In silviculture, removal of lower branches improves the quality of the bole for lumber, but the effect on growth would depend to a large extent on whether or not the pruned branches were parasitic on the upper crown. The present work indicates that, as with agronomic crops, there is no simple answer to this question. For example, over a full day, some of the foliage in the lower branch whorls might well have constituted a net respiratory load on their branches, particularly the 4-year-old foliage in the 1961-3 spring whorls (Appendix 9, Table A44). On the other hand, it is unlikely that the branches as a whole were parasitic on the upper crown because a net respiratory loss by the older foliage probably would have been balanced by a net photosynthetic gain in the younger foliage which they also carried. Presumably a fine balance is achieved. This is supported by the repeated observation that growth does not seem to be affected either way

when up to 30 per cent of the green crown is removed in pruning branches from the crown base in many conifers, e.g., P. patula, P. caribaea, P. taeda (Lückhoff, 1949); Pseudotsuga sp. (Stein, 1955); P. radiata (Shepherd, 1961, 1967). To answer the question fully, more information is required.

Silvicultural treatment of a forest stand frequently alters factors of the forest environment which, in turn, affect tree development. One good example is the better development of branches on trees in thinned than in unthinned stands. This type of compensatory response is similar to that observed with increased lateral branching of seedlings growing under high intensity light in the 'CERES' phytotron (see p.160). Presumably the increased branch development in thinned stands is related to the increased light intensity following thinning. In addition to light responses, the laboratory studies on seedlings indicate that temperature, soil moisture, and shoot severance evoke responses which appear to be of a similar compensatory nature. In the same way that light intensity appears to have similar effects on the branches of seedlings and trees, it is tempting to speculate that other responses to light and responses to temperature and soil moisture may also be similar in seedlings and older plants. However, evidence suggests that speculation along these lines is not worthwhile, e.g., low light conditions correspond with increased pigment concentration and increased photosynthetic activity in leaves of seedlings (Appendix 5, Tables A23, A24, and A32) but the converse

relationship was found in the leaves of a plantation sapling (Appendix 8, Tables A40, A42; and Appendix 9, Table A44). Accordingly, the several compensatory responses observed in the seedling studies may likewise not apply to trees in plantations, and no extrapolation of them is attempted.

Responses of seedlings to the light environment vary according to the intensity of light. Under low light conditions, both morphological and physiological responses of a compensatory nature were found. For example, the increased height, reduced branching, widely spaced leaves on the stem, and high leaf angles of seedlings raised under shade are all features which are expected to reduce self-shading and improve the display of foliage either separately or in combination; and this improves the capacity of leaves to intercept light. As regards physiological responses to low light, the high concentration of plant pigments (Appendix 5, Tables A23, A24) suggests efficient light absorption, while the low Ca/Cb ratio (Appendix 5, Table A25) implies increased levels of chlorophyll-b relative to chlorophyll-a which might improve the efficiency of light absorption in green shade light. An additional feature of the shade grown seedlings was the high proportion of functional primary leaves. These leaves are very efficient photosynthetically in Pinus taeda (Bormann, 1958), and there is no reason to suppose that they are not equally efficient in Pinus radiata. All these features might well explain the high capacities of the photochemical process and high rates of net photosynthesis observed on shade grown seedlings (Appendix 5, Tables A30, A32).

However, the compensatory value of the responses to high intensity light seems doubtful. Both increased heating and drying are involved. The heating effect would probably be lessened by the low leaf angle (Appendix 5, Table A9) which tends to reduce the amount of radiant energy absorbed by a plant. The high density of foliage and heavy branching (Appendix 5, Tables A17-A19) would increase the level of self-shading and this might lessen the effects of drying by reducing the rate of transpiration (Appendix 5, Table A33). These secondary effects of high light intensity cannot be clearly separated from the primary effects. Though leaves tend to be more erect under high intensity light, a concomitant high temperature would increase leaf angle (Appendix 5, Table A9).

As with light, responses of seedlings to temperature vary depending on whether levels are high or low. Both heat and water stresses are associated with high temperature, and some of the morphological and physiological responses of P. radiata seedlings to these stresses appear compensatory. The reduced number of fascicles per unit length of stem and reduced branching at 33/28°C (Appendix 5, Tables A17 and A18/A19 respectively) are features which ensure a more open crown with an associated increase in the air turbulence amongst the foliage and reduction of boundary layer resistance. It is very likely, therefore, that heat exchange will be facilitated by these responses to high temperature stress. Another high temperature response is the increased proportion of fascicles carrying 2 instead of the usual 3 needles

(Appendix 5, Table A12). Associated with this response is the reduced surface area of needles per fascicle and thus reduced interception of radiant energy and exchange of gases. Again, both the heat load on the foliage and the rate of water loss are apparently reduced by the compensatory responses, although the decreased water loss presumably may be outweighed by increased transpiration associated with an increased turbulence in the more open crown. Concomitant decreases in photosynthesis will be expected with any decrease in gas exchange. On the other hand, the high concentration of leaf pigment at high temperatures (Appendix 5, Tables A23, A24) would be expected to improve the photosynthetic capacity of leaves and compensate in part for the reduced surface area of needles per fascicle.

Although no obvious stress is involved in the photosynthesis of seedlings at 11°C when light is limiting, there is evidence of stress under non-limiting light (Figure 3). The increased proportion of fascicles carrying 4 and 5 needles on seedlings grown at 15/10°C (Appendix 5, Table A13) could be interpreted as a compensatory response to low temperature when light is non-limiting. Under these conditions, the increased needle surface area of the higher order fascicles would be important not so much in increasing the interception of light but in increasing the exchange of gases at the low temperatures.

The depression in the capacity of the photochemical process of photosynthesis at 35°C (Table 3) indicates the breakdown or reduction of possible compensatory

responses near this temperature. The reduced dry matter production at 33/28°C in the 'CERES' phytotron (Appendix 5, Table A37) supports this interpretation. Reduced compensatory responses at high temperatures may be involved in the summer 'dormancy' often observed in P. radiata plantations in Australia. Denmead (1966) has raised the interesting point that a markedly reduced photosynthesis in summer may be associated with such summer 'dormancy'. Foresters commonly observe a marked decrease in tree growth during summer but generally attribute the phenomenon to water stress. However, high temperatures obviously should not be overlooked as a possible causal factor, particularly in view of the observations summarised by Scott (1960) which suggest that P. radiata does not easily withstand extreme heat. Again, speculation along these lines is of doubtful value when it involves extrapolation from seedling behaviour to growth of the older tree. However, it is interesting to note that the moderate temperatures (low twenties) and high light intensities which favour photosynthesis and growth in seedlings (Figure 3; Appendix 5, Table A37) are similar to those which are claimed to be essential for optimum growth of the species in the field (Pawsey, 1953; Ruiter, 1966).

Compensatory responses of P. radiata seedlings to light and temperature are thus most obvious under the stress conditions of low light and high temperature respectively. In contrast, they are equally obvious under conditions of soil water deficit and excess.

As soil moisture content decreases, a stage is reached when the water deficit in leaves results in closure of the stomata thus restricting passage of CO_2 into the leaves. Because water stress in the leaf is the result of differences between water uptake and loss, it is expected that the rate at which a plant reaches the point of stomatal closure will depend on the nature and dryness of the soil and the evaporative demand. If stomatal closure occurs at a RWC at which the metabolic activity of cells is disrupted, then the ability to close stomata and reduce the rate of onset of more severe water stress is of limited value. Thus, the higher the RWC at which a plant closes its stomata, the more it is able to conserve moisture. P. radiata needles have been found to sustain inner tissue damage at RWCs as high as 75 per cent (Oppenheimer, 1968). Therefore, it is expected that stomatal closure at RWCs in the low eighties, as is indicated by the steep fall in photosynthesis to negligible proportions (Figures 10, 11, 17; Appendix 9, Table A44), will protect the leaf tissue from damage. However, as P. radiata needles have been found to have a comparatively high cuticular transpiration rate (Oppenheimer, op. cit.), the RWC of the leaf will continue to fall if drought conditions are maintained. This is indicated by measurements of RWC as low as 40 per cent (Figure 10). Evidence of damage to the photosynthetic mechanism is probably indicated in Figure 11 where the recovery of photosynthetic activity following the severe third drought cycle (RWC approximately 50 per cent) was delayed several days

after removal of the plant water deficit. On the other hand, recovery was rapid following watering after the first and second cycles of drought during which the RWCs were not as severely reduced (Figure 11).

Water stress may also occur in a plant under saturated soil conditions. Indirect evidence suggests that the reduction observed in the rates of net photosynthesis and transpiration in the period immediately after flooding (Figure 12) was probably caused by a plant water deficit arising from reduced water absorption (Figure 18). A compensatory response by the plant is evidenced by the fact that after a steep initial reduction in photosynthesis and transpiration to approximately 60 per cent of normal (Figures 12 and 13), it was able to maintain its rates of gas exchange at approximately this level for 4-5 weeks (Figure 13). The manner in which this was achieved is not known, but there is evidence with other plant species that diffusion of oxygen through the stem to the root zone may be involved (Kursanov, 1966; Greenwood, 1967a, b; Letey and Stolzy, 1967).

In spite of the observed compensatory responses of seedlings to soil water deficit and excess, there is a limit to which seedlings can withstand these conditions (Figures 10 and 13). Observations suggest that the RWC of needles lethal to P. radiata seedlings may lie between 40-50 per cent (Figures 10 and 11). RWCs within this range are lethal to many plants (Jarvis, 1963; Jarvis and Jarvis, 1963; Pharis, 1966; Pook et al., 1966). It would be worthwhile determining whether the lethal RWC and the threshold RWC for stomatal closure

can be defined reliably for the species. Such information would have immediate practical value in nursery irrigation and in assessment of the physiological condition of trees during drought.

Many of the results related to compensatory responses suggest reasons why it is possible for P. radiata to exist under a wide and diverse range of environments. However, it would be necessary to determine whether or not these responses are usual in the genus as a whole before suggesting that they provide an explanation, at least in part, for the outstanding performance of Pinus radiata as a plantation exotic in many countries.

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APPENDIX I

ESTIMATING THE SURFACE AREA AND VOLUME OF A P. RADIATA NEEDLE

(a) The cross-sectional and solid shape of a ternate P. radiata fascicle

Kozlowski and Schumacher (1943) observed that the outline of any cross-section along the length of a fascicle of P. taeda and P. strobus was circular, and that the contiguous faces of needles, as viewed in cross-section, could be regarded as radii of the fascicle circle. Minckler (1939) observed in P. strobus that the taper along the needle length was very small. These observations implied that the solid shape of the fascicle was approximately cylindrical.

Madgwick (1964) found with P. resinosa needles that the assumption of cylindrical shape of a fascicle was the best approximation to actual shape, and that estimates of needle surface area based on it lead to slight errors only (average of +6 per cent). If a similar assumption could be made with P. radiata fascicles, the estimation of needle surface area would be facilitated.

As a preliminary trial, a single ternate fascicle of P. radiata, 10 cm long from the fascicle sheath, was sectioned at the top of the sheath (0 cm), and at 2, 4, 6, and 8 cm using a sliding microtome set at 20μ. The orientation of needles within sections was carefully

preserved. The sections were mounted in water, and the widths of the radial faces of each, as well as needle thickness, were measured under the microscope using an eye piece micrometer attachment which had previously been calibrated against a stage micrometer.

Results are presented in Table A1.

TABLE A1 Variation in the cross-sectional shape along the length of a ternate P. radiata fascicle

Section no.	Distance from fascicle sheath (cm)	R* (mm)
1	0	0.806 ± 0.032
2	2	0.839 ± 0.023
3	4	0.898 ± 0.012
4	6	0.904 ± 0.011
5	8	0.870 ± 0.012

* R is the average of nine measurements (6 sides and 3 thicknesses) made on the three needle sections comprising each fascicle section.

The small standard error of R for each fascicle section indicates that the fascicle shape is approximately circular throughout its length. Also, the comparatively small variation in the value of R along the length of the fascicle suggests that the solid shape of the fascicle is approximately cylindrical. The shape was examined in greater detail by measuring the thickness of each of 12 needles at 10 equidistant points along their length. Results are presented in Table A2.

TABLE A2 Variation in the thickness of P. radiata needles along their length expressed as a percentage of the maximum thickness observed per needle

Percentile point	Thickness
0	80.3 ± 1.5
10	87.0 ± 1.5
20	91.8 ± 1.3
30	94.4 ± 0.8
40	97.6 ± 0.8
50	98.7 ± 0.6
60	97.4 ± 0.6
70	96.3 ± 0.6
80	96.0 ± 0.9
90	88.0 ± 1.1
100 (Tip)	-

Table A2 indicates that the variation in the thickness of needles along their length is small. Thus, one can assume that the solid shape of a P. radiata fascicle is approximately cylindrical. Any estimation of needle surface area or volume based on this assumption will lead to a positive error which should be roughly comparable with that found by Madgwick (op. cit.).

(b) Estimating the surface area of a P. radiata needle from a ternate fascicle

Let R represent the width of the radial faces of a needle sectioned in the middle and let L represent total needle length.

Perimeter of needle = R + R + 1/3 circumference

$$= 2R + \frac{2\pi R}{3}$$

$$= 2R\ (1 + 1.047)$$

$$= 4.094R$$

whence surface area $\approx 4.1LR$

and volume $= (\frac{\pi R^2}{3})L$

$$= 1.047R^2L$$

APPENDIX 2

VARIATION IN THE NUMBER OF STOMATAL ROWS ALONG THE LENGTH OF A P. RADIATA NEEDLE

Some knowledge of the extent of variation in the number of stomatal rows along the length of a needle is essential if one proposes to investigate the variation in the parameter between needles of a given tree or of a number of trees (see Kriebel and Fowler, 1965). Sampling obviously should be confined to that section of a needle which shows least variation in the number of rows along its length.

Twelve 1-year-old needles were detached from the upper crown of a 6-year-old tree, and the number of stomatal rows on the curved abaxial faces was counted at 10 equidistant points along the length of each. Results are presented in Table A3.

Table A3 indicates that the least variability in the number of stomatal rows on the abaxial face of a needle occurs at mid-length. The maximum number of rows also occurs in this area.

It was observed during the study described above that stomatal rows along the radial and abaxial faces of a needle appear and disappear at random (Figure A1). The absence of stomata from the basal 1-2 cm of the radial faces (and the lower chlorophyll content in this section of needle - Wood, 1967) suggests that the CO_2-exchange capacity at the base of the needle will

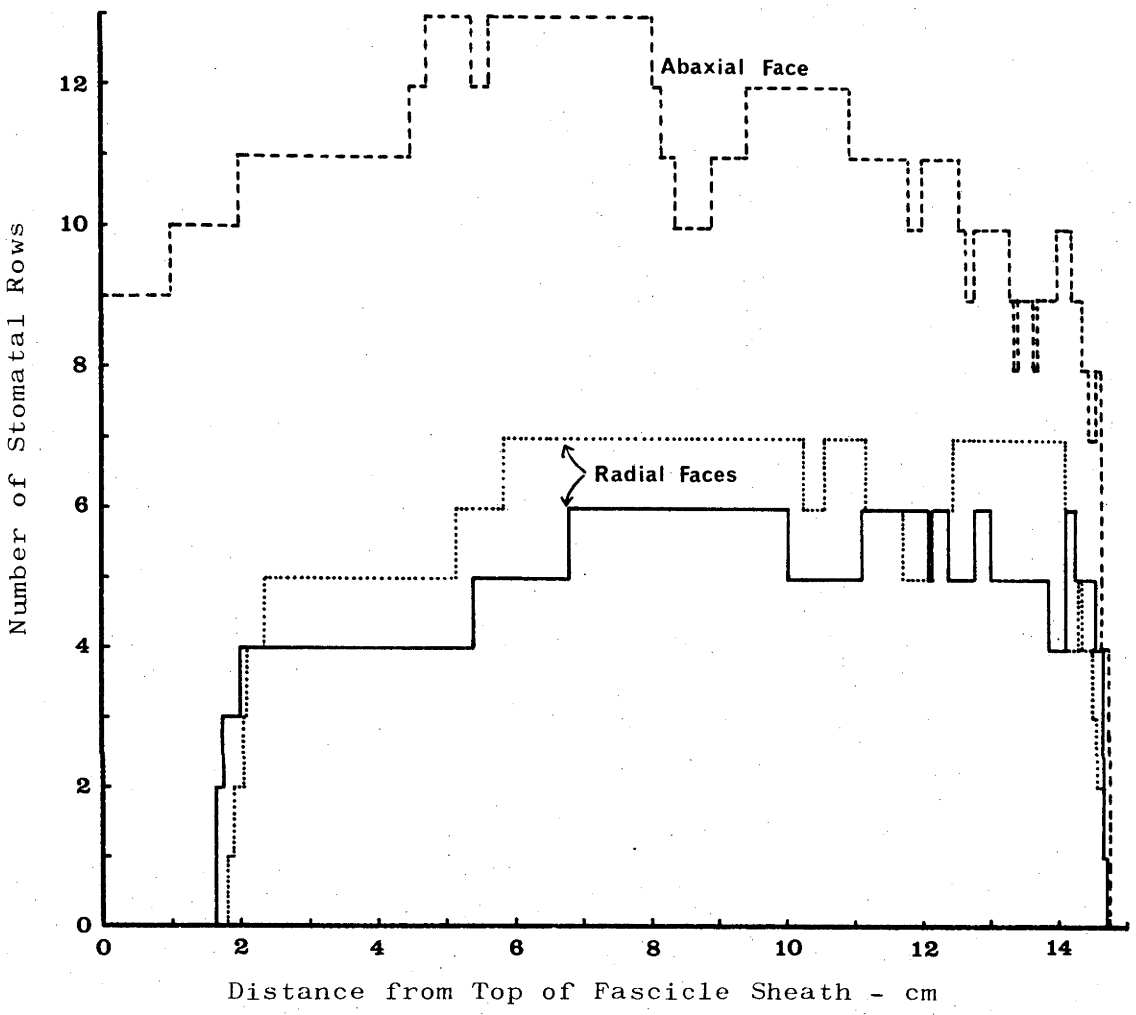

FIGURE **A1 :** Variation in the number of stomatal rows on the abaxial and inner faces of a P. radiata needle 14.8 cm long from the top of the fascicle sheath.

TABLE A3 Variation in the number of stomatal rows at the 10-percentile points along the abaxial faces of 1-year-old P. radiata needles. Results are expressed as a percentage of the maximum number of rows observed on each needle

Percentile point	No. of stomatal rows (% of observed maximum)
0 (Top of sheath)	81.4 ± 4.6
10	90.7 ± 2.6
20	94.9 ± 1.9
30	94.5 ± 1.7
40	96.0 ± 1.5
50	95.7 ± 1.6
60	95.5 ± 1.7
70	95.6 ± 1.5
80	91.6 ± 1.5
90	86.6 ± 2.4
100 (Tip)	-

be lower than that at other points along its length, with the possible exception of the extreme tip where chlorophyll levels are also low (Wood, op. cit.).

APPENDIX 3

THE PREPARATION OF MATERIAL AND THE EXTRACTION AND SPECTROPHOTOMETRIC ANALYSIS OF PIGMENTS IN *P. RADIATA* NEEDLES

Preparation of material: Sampling of plant pigments was confined to the mid-section of mature needles. This was based on the finding of Wood (1967) that the middle third of fully expanded needles is the most reliable position in which to sample for pigment in *P. radiata*.

Extraction of plant pigments: The procedure outlined below is based on methods for quantitative extraction described by Mackinney (1941) and Arnon (1949).

(i) The fresh sample material was macerated with a mortar and pestle and, after washing with 80 per cent acetone (acetone/distilled water - v/v), the macerated material and solvent were transferred to a centrifuge tube.

(ii) Centrifuged at 3000 r.p.m. for three minutes.

(iii) Decanted off the supernatant into a 25 ml volumetric flask.

(iv) Added more solvent to the centrifuge tube and repeated steps (ii) and (iii) until extraction was complete.

(v) Added 80 per cent acetone to the volumetric flasks to bring them to volume.

(vi) Stored the pigment extracts in the dark at 2°C until required for spectrophotometric analysis. The longest period of storage was 48 hours. No deterioration or isomerisation of chlorophyll would occur in this short period under these storage conditions (Zscheile et al., 1944; Wood, 1967).

Note: The dry weight of the material extracted was determined from the known fresh weight and the calculated moisture content of a duplicate sample taken from companion needles in the sample fascicles, i.e., of the three needles in a fascicle, one was used for pigment analysis and the other two for the determination of moisture content.

Spectrophotometric analysis: Spectrophotometric analysis was conducted on three replicates of each sample at 450, 645, and 663 mμ. The recommendations of Mellon (1950), Smith and Benitez (1955), and Glover (1956) were followed in the spectrophotometric procedure to minimise sources of error. The concentrations of chlorophyll-a, chlorophyll-b, and chlorophyll-a+b were determined using the formulae of Arnon (1949), and the concentration of carotenoids using the formula of Jaspars (1965).

APPENDIX 4

PROCEDURE USED IN MEASURING THE RELATIVE WATER CONTENT OF P. RADIATA NEEDLES

(1) Time of sampling - This varied with the experiment. Where a measure of the RWC at different positions in the canopy of a forest tree was sought, sampling was done at dawn. In cases where RWC data were required to interpret the CO_2- and H_2O-vapour exchange responses of a plant, the RWC measurement was conducted at the time of gas exchange measurement.

(2) Sample size - 0.5 to 1.0g fresh weight. Duplicate samples were taken in all cases.

(3) Storage time prior to saturation - Storage was unnecessary. Fascicles were detached from the plant and, after severing the needles with a sharp razor blade at a point just above the level of the fascicle sheath, they were transferred directly to tared, tightly stoppered, 200 mm x 25 mm test tubes containing 1 ml of distilled water. The fresh weight of samples was then determined to 0.1mg.

(4) Saturation procedure - The test tubes containing the needle samples were transferred to an incubator and allowed to saturate in the dark for 24 hours. The incubator temperature varied with the experiment, but was generally $25 \pm 1^{\circ}C$ as most gas exchange work was done at this temperature.

(5) Turgid weight determination - After saturation, the samples were removed from the test tubes, surface dried with absorbent tissue, and quickly transferred to another set of tared, tightly stoppered test tubes. The turgid weight was then determined to 0.1mg.

(6) Oven dry weight determination - The needle samples were placed in an oven at 95°C for 48 hours, and after cooling in a desiccator, were then weighed to 0.1mg.

(7) Calculation of relative water content -

$$\text{RWC } (\%) = \left(\frac{\text{fresh weight - oven dry weight}}{\text{turgid weight - oven dry weight}}\right) \times 100$$

The procedure outlined above is based on methods described by Johnston (1957), Rutter and Sands (1958), Harms and McGregor (1962), Clausen and Kozlowski (1965b), and Shepherd (1965), all of which are modifications of Weatherley's relative turgidity technique (Weatherley, 1950), fitting it for use with coniferous species. The modification of Millar (1966a, b), designed to minimise an error due to temperature effects, has been incorporated.

APPENDIX 5

SECTION II : STUDY 2 - DATA

A. Needle and fascicle morphology : 8-month-old P. radiata seedlings

TABLE A4 Needle length (cm)

Temperature regime (°C)	Per cent Shading			Mean (±0.5)
	0	46 (±0.9)	74	
15/10	12.2	11.9	12.4	12.2
21/16	13.8	14.3	14.8	14.3
27/22	15.4	15.7	17.2	16.1
33/28	17.1	15.0	12.4	14.8
Mean (±0.4)	14.6	14.2	14.2	14.4
	L.S.D.5% 2.6	L.S.D.1% 3.7	L.S.D. .1% 5.2	

TABLE A5 Needle thickness (mm)

Temperature regime (°C)	Per cent Shading			Mean (±0.02)
	0	46 (±0.03)	74	
15/10	0.76	0.60	0.50	0.62
21/16	0.72	0.63	0.56	0.64
27/22	0.64	0.56	0.58	0.59
33/28	0.56	0.55	0.44	0.52
Mean (±0.02)	0.67	0.58	0.52	0.59
	L.S.D.5% 0.09	L.S.D.1% 0.13	L.S.D. .1% 0.19	

APPENDIX 5 (cont.)

TABLE A6 Needle shape (= length/thickness ratio)

Temperature regime (°C)	Per cent Shading			Mean (±11.0)
	0	46 (±19.0)	74	
15/10	160.5	200.0	246.5	202.3
21/16	190.5	228.0	263.5	227.3
27/22	238.5	283.5	298.0	273.3
33/28	307.5	272.5	283.0	287.7
Mean (±9.5)	224.2	246.0	272.8	247.7
	L.S.D.5% 58.5	L.S.D.1% 82.1	L.S.D. .1% 116.0	

TABLE A7 Needle surface area (cm^2)

Temperature regime (°C)	Per cent Shading			Mean (±0.18)
	0	46 (±0.31)	74	
15/10	3.72	2.83	2.50	3.02
21/16	4.00	3.61	3.31	3.64
27/22	3.96	3.50	4.04	3.83
33/28	3.83	3.30	2.19	3.11
Mean (± 0.15)	3.88	3.31	3.01	3.40
	L.S.D.5% 0.10	L.S.D.1% 0.13	L.S.D. .1% 0.19	

APPENDIX 5 (cont.)

TABLE A8 Length of fascicle sheath (cm)

Temperature regime (°C)	Per cent Shading			Mean (±0.0)
	0	46 (±0.1)	74	
15/10	1.0	1.3	1.3	1.2
21/16	1.2	1.1	1.0	1.1
27/22	1.0	1.1	0.9	1.0
33/28	0.7	1.0	0.8	0.8
Mean (±0.0)	1.0	1.1	1.0	1.0
	L.S.D.5% 0.2	L.S.D.1% 0.3	L.S.D. .1% 0.4	

TABLE A9 Fascicle angle (degrees)

Temperature regime (°C)	Per cent Shading			Mean (± 2)
	0	46 (± 3)	74	
15/10	46	46	46	46
21/16	53	77	80	70
27/22	67	83	84	78
33/28	76	80	80	79
Mean (± 2)	61	72	73	68
	L.S.D.5% 9	L.S.D.1% 13	L.S.D. .1% 17	

APPENDIX 5 (cont.)

TABLE A10 Number of stomatal rows on abaxial face of needle

Temperature regime (°C)	Per cent Shading			Mean (±0.2)
	0	46 (±0.4)	74	
15/10	7.5	6.0	5.2	6.2
21/16	8.2	7.8	7.2	7.7
27/22	8.7	8.2	7.5	8.2
33/28	8.7	9.0	7.1	8.3
Mean (±0.3)	8.3	7.8	6.7	7.6
	L.S.D.5% 1.2	L.S.D.1% 1.7	L.S.D. .1% 2.4	

TABLE A11 Stomatal rows on abaxial face per mm of needle thickness

Temperature regime (°C)	Per cent Shading			Mean (±0.5)
	0	46 (±0.9)	74	
15/10	9.8	10.1	10.2	10.0
21/16	11.4	12.4	12.8	12.2
27/22	13.5	15.0	13.0	13.8
33/28	15.6	16.3	16.2	16.0
Mean (±0.5)	12.6	13.4	13.0	13.0
	L.S.D.5% 2.8	L.S.D.1% 4.0	L.S.D. .1% 5.6	

APPENDIX 5 (cont.)

TABLE A12 Occurrence of 3-needled fascicles (per sample of 25 fascicles)

Temperature regime (°C)	Per cent Shading			Mean (±0.7)
	0	46 (±1.3)	74	
15/10	3.5	7.0	14.0	8.2
21/16	22.0	20.5	20.0	20.8
27/22	23.0	24.5	23.5	23.7
33/28	20.5	21.0	18.5	20.0
Mean (±0.8)	17.2	18.2	19.0	18.2
	L.S.D.5% 4.0	L.S.D.1% 5.7	L.S.D. .1% 8.0	

Note: Some 2-needled fascicles occurred in shade treatments under the 33/28°C regime

TABLE A13 Occurrence of 4-needled fascicles (per sample of 25 fascicles)

Temperature regime (°C)	Per cent Shading			Mean (±0.5)
	0	46 (±1.1)	74	
15/10	17.5	15.5	11.0	14.7
21/16	3.0	4.5	5.0	4.2
27/22	2.0	0.5	1.5	1.3
33/28	3.0	3.5	0.5	2.3
Mean (±0.6)	6.4	6.0	4.5	5.6
	L.S.D.5% 3.3	L.S.D.1% 4.6	L.S.D. .1% 6.5	

Note: Some 5-needled fascicles occurred in shade treatments under the 15/10°C regime.

APPENDIX 5 (cont.)

B. Plant morphology : 8-month-old P. radiata seedlings

TABLE A14 Seedling height (cm)

Temperature regime (°C)	Per cent Shading			Mean (±1.4)
	0	46 (±2.4)	74	
15/10	42.9	50.0	46.4	46.4
21/16	72.6	79.9	69.2	73.9
27/22	75.4	71.6	55.4	67.4
33/28	60.5	72.3	57.8	63.5
Mean (±1.2)	62.8	68.4	57.2	62.8
	L.S.D.5% 6.9	L.S.D.1% 9.1	L.S.D. .1% 11.9	

TABLE A15 Basal stem diameter (cm)

Temperature regime (°C)	Per cent Shading			Mean (±0.01)
	0	46 (±0.02)	74	
15/10	0.83	0.65	0.48	0.66
21/16	0.93	0.78	0.58	0.76
27/22	0.94	0.70	0.49	0.71
33/28	0.74	0.59	0.39	0.57
Mean (±0.01)	0.86	0.68	0.49	0.67
	L.S.D.5% 0.07	L.S.D.1% 0.09	L.S.D. .1% 0.12	

APPENDIX 5 (cont.)

TABLE A16 Height/diameter ratio

Temperature regime (°C)	Per cent Shading			Mean (±1.8)
	0	46 (±3.1)	74	
15/10	51.4	77.0	96.3	74.9
21/16	77.8	102.9	120.8	100.5
27/22	80.6	103.0	111.8	98.5
33/28	82.6	123.6	149.0	118.4
Mean (±1.6)	73.1	101.6	119.5	98.1
	L.S.D.5% 8.7	L.S.D.1% 11.6	L.S.D. .1% 15.1	

TABLE A17 Number of fascicles/cm of stem length

Temperature regime (°C)	Per cent Shading			Mean (±0.1)
	0	46 (±0.2)	74	
15/10	5.8	4.1	3.2	4.4
21/16	5.2	4.6	3.8	4.5
27/22	4.3	3.5	2.7	3.5
33/28	4.2	2.5	2.1	2.9
Mean (±0.1)	4.9	3.6	3.0	3.8
	L.S.D.5% 0.7	L.S.D.1% 0.9	L.S.D. .1% 1.1	

APPENDIX 5 (cont.)

TABLE A18 Number of long shoots per stem

Temperature regime (°C)	Per cent Shading			Mean (±0.4)
	0	46 (±0.6)	74	
15/10	9.1	6.1	5.2	6.8
21/16	7.1	5.8	5.4	6.1
27/22	6.4	5.8	5.5	5.9
33/28	6.4	5.9	4.2	5.5
Mean (±0.3)	7.2	5.9	5.1	6.1
	L.S.D.5% 1.8	L.S.D.1% 2.3	L.S.D. .1% 3.1	

TABLE A19 Ratio - O.D.Wt. long shoots/O.D.Wt. whole stem

Temperature regime (°C)	Per cent Shading			Mean (±0.02)
	0	46 (±0.03)	74	
15/10	0.30	0.20	0.14	0.21
21/16	0.17	0.10	0.09	0.12
27/22	0.16	0.09	0.03	0.09
33/28	0.21	0.06	0.06	0.11
Mean (±0.02)	0.21	0.11	0.08	0.13
	L.S.D.5% 0.09	L.S.D.1% 0.12	L.S.D. .1% 0.16	

APPENDIX 5 (cont.)

TABLE A20 Plant stockiness

Temperature regime (°C)	Per cent Shading			Mean (±0.02)
	0	46 (±0.03)	74	
15/10	0.50	0.29	0.18	0.33
21/16	0.50	0.34	0.19	0.34
27/22	0.46	0.24	0.12	0.27
33/28	0.28	0.17	0.07	0.17
Mean (±0.02)	0.43	0.26	0.14	0.28
	L.S.D.5% 0.09	L.S.D.1% 0.13	L.S.D. .1% 0.17	

C. Needle physiology : 8-month-old P. radiata seedlings

TABLE A21 Concentration of chlorophyll-a (mg/g O.D.Wt.)

Temperature regime (°C)	Per cent Shading			Mean (±0.13)
	0	46 (±0.23)	74	
15/10	2.52	4.17	4.51	3.74
21/16	2.33	4.02	4.72	3.69
27/22	3.04	4.46	5.31	4.27
33/28	3.29	4.43	4.82	4.18
Mean (±0.12)	2.79	4.27	4.84	3.97
	L.S.D.5% 0.72	L.S.D.1% 1.01	L.S.D. .1% 1.42	

APPENDIX 5 (cont.)

TABLE A22 Concentration of chlorophyll-b (mg/g O.D.Wt.)

Temperature regime (°C)	Per cent Shading			Mean (±0.05)
	0	46 (±0.09)	74	
15/10	0.87	1.55	1.72	1.38
21/16	0.89	1.62	1.97	1.49
27/22	1.44	2.06	2.49	2.00
33/28	1.52	2.04	2.34	1.97
Mean (±0.05)	1.18	1.82	2.13	1.71
	L.S.D.5% 0.29	L.S.D.1% 0.41	L.S.D. .1% 0.58	

TABLE A23 Concentration of chlorophyll-a+b (mg/g O.D.Wt.)

Temperature regime (°C)	Per cent Shading			Mean (±0.19)
	0	46 (±0.32)	74	
15/10	3.39	5.72	6.23	5.12
21/16	3.22	5.64	6.68	5.18
27/22	4.48	6.52	7.80	6.27
33/28	4.81	6.47	7.16	6.15
Mean (±0.16)	3.98	6.09	6.97	5.68
	L.S.D.5% 0.99	L.S.D.1% 1.39	L.S.D. .1% 1.97	

APPENDIX 5 (cont.)

TABLE A24 Concentration of carotenoids (mg/g O.D.Wt.)*

Temperature regime (°C)	Per cent Shading			Mean (±0.09)
	0	46 (±0.16)	74	
15/10	1.79	2.00	2.22	2.00
21/16	2.06	2.68	2.58	2.44
27/22	2.02	2.71	3.02	2.58
33/28	2.01	2.78	3.32	2.70
Mean (±0.08)	1.97	2.53	2.79	2.43
	L.S.D.5% 0.50	L.S.D.1% 0.70	L.S.D. .1% 0.99	

* Data obtained in a duplicate (repeat) study

TABLE A25 Ca/Cb ratio

Temperature regime (°C)	Per cent Shading			Mean (±0.03)
	0	46 (±0.05)	74	
15/10	2.89	2.70	2.62	2.74
21/16	2.62	2.48	2.40	2.50
27/22	2.11	2.12	2.12	2.13
33/28	2.18	2.18	2.10	2.14
Mean (±0.03)	2.45	2.38	2.30	2.38
	L.S.D.5% 0.16	L.S.D.1% 0.22	L.S.D. .1% 0.31	

APPENDIX 5 (cont.)

TABLE A26 Ca+b/carotenoid ratio$^{\phi}$

Temperature regime (°C)	Per cent Shading			Mean (±0.08)
	0	46 (±0.14)	74	
15/10	3.02	3.16	3.22	3.14
21/16	3.28	3.32	3.28	3.29
27/22	3.60	3.48	3.29	3.46
33/28	3.27	3.16	3.22	3.29
Mean (±0.07)	3.29	3.28	3.25	3.28
	L.S.D.5% 0.43	L.S.D.1% 0.60	L.S.D. .1% 0.85	

ϕ Data obtained in a duplicate (repeat) study

TABLE A27 Needle moisture content (% O.D.Wt.)

Temperature regime (°C)	Per cent Shading			Mean (± 9)
	0	46 (±16)	74	
15/10	225	255	234	238
21/16	191	227	250	222
27/22	186	238	238	221
33/28	179	240	225	214
Mean (± 8)	195	240	236	224
	L.S.D.5% 50	L.S.D.1% 70	L.S.D. .1% 99	

APPENDIX 5 (cont.)

TABLE A28 Rate of dark respiration (mg CO_2/c.c./hr)*

Temperature regime (°C)	Per cent Shading			Mean (±0.01)
	0	46 (±0.02)	74	
15/10	0.24	0.22	0.17	0.21
21/16	0.23	0.17	0.13	0.18
27/22	0.32	0.22	0.23	0.25
33/28	0.35	0.23	0.23	0.27
Mean (±0.01)	0.28	0.21	0.19	0.23
	L.S.D.5% 0.07	L.S.D.1% 0.10	L.S.D. .1% 0.13	

* The rate of dark respiration was recorded at the day temperature, viz., 15°C for the 15/10°C regime; 21°C for the 21/16°C regime; etc.

TABLE A29 Compensation point for light (ft.c.)ø

Temperature regime (°C)	Per cent Shading			Mean (±19)
	0	46 (±32)	74	
15/10	238	173	117	176
21/16	323	142	83	183
27/22	440	252	183	292
33/28	417	217	177	270
Mean (± 16)	355	196	140	230
	L.S.D.5% 94	L.S.D.1% 127	L.S.D. .1% 170	

ø Light response curves were recorded for the day temperature, viz., at 15°C in the 15/10°C regime; 21°C in the 21/16°C regime; etc.

APPENDIX 5 (cont.)

TABLE A30 Initial slope of light response curve*

Temperature regime (°C)	Per cent Shading			Mean (±0.08)
	0	46 (±0.13)	74	
15/10	1.99	2.62	2.95	2.52
21/16	1.42	2.41	3.01	2.28
27/22	1.41	1.73	2.51	1.88
33/28	1.73	2.06	2.35	2.05
Mean (±0.07)	1.64	2.21	2.71	2.18
	L.S.D.5% 0.39	L.S.D.1% 0.53	L.S.D. .1% 0.71	

* Light response curves were recorded for the day temperature, viz., in the 15/10°C regime at 15°C; in the 21/16°C regime at 21°C; etc.

TABLE A31 P3500/R ratio$^{\phi}$

Temperature regime (°C)	Per cent Shading			Mean (±0.8)
	0	46 (±1.3)	74	
15/10	8.5	11.2	16.9	12.2
21/16	6.3	13.3	17.8	12.5
27/22	2.3	9.2	10.3	7.3
33/28	4.6	8.1	6.9	6.5
Mean (±0.7)	5.4	10.4	13.0	9.6
	L.S.D.5% 3.9	L.S.D.1% 5.3	L.S.D. .1% 7.1	

ϕ Where P3500 = rate of gross photosynthesis at 3500 ft.c. = rate of net photosynthesis at 3500 ft.c. + dark respiration rate (R)

Note: P and R were measured at the day temperature, viz., in the 15/10°C regime at 15°C; in the 21/16°C regime at 21°C; etc.

APPENDIX 5 (cont.)

TABLE A32 Rate of net photosynthesis (mg CO_2/c.c./hr) of foliage at 500, 1500, 2500, and 3500 ft.c. in 8- to 9-month-old P. radiata seedlings grown in a controlled environment under a range of temperature and shading regimes*

Illumination (ft.c.)	Temperature regime (°C)	Per cent Shading			Mean	L.S.D. 5%
		0	46	74		
			(±0.04)		(±0.02)	
	15/10	0.26	0.43	0.57	0.42	
	21/16	0.13	0.43	0.62	0.39	
500	27/22	0.04	0.20	0.40	0.21	0.12
	33/28	0.08	0.30	0.37	0.25	
	Mean (±0.02)	0.13	0.34	0.49	0.32	
			(±0.10)		(±0.06)	
	15/10	1.11	1.57	1.87	1.52	
	21/16	0.79	1.40	1.80	1.33	
1500	27/22	0.36	1.03	1.49	0.96	0.29
	33/28	0.77	1.16	1.11	1.02	
	Mean (±0.05)	0.76	1.29	1.57	1.21	
			(±0.11)		(±0.07)	
	15/10	1.61	2.07	2.34	2.01	
	21/16	1.05	1.92	2.06	1.68	
2500	27/22	0.44	1.58	1.88	1.30	0.34
	33/28	1.10	1.49	1.33	1.31	
	Mean (±0.06)	1.05	1.76	1.90	1.57	
			(±0.11)		(±0.05)	
	15/10	1.80	2.27	2.55	2.21	
	21/16	1.12	2.12	2.10	1.78	
3500	27/22	0.43	1.76	2.04	1.41	0.32
	33/28	1.19	1.56	1.35	1.37	
	Mean (±0.06)	1.14	1.93	2.01	1.69	

* The rate of net P was measured at the day temperature, viz., in the 15/10°C regime at 15°C; in the 21/16°C regime at 21°C; etc.

APPENDIX 5 (cont.)

TABLE A33 Rate of transpiration* (mg H_2O/c.c./hr) of foliage at 500, 1500, 2500, and 3500 ft.c. in 8-to 9-month-old P. radiata seedlings grown in a controlled environment under a range of temperature and shading regimes

Illumination (ft.c.)	Temperature regime (°C)	Per cent Shading			Mean	L.S.D. 5%
		0	46	74		
			(±29.4)		(±17.0)	
	15/10	26.2	77.7	89.4	64.4	
	21/16	8.5	164.7	125.1	99.5	
500	27/22	1.2	40.9	116.0	52.7	85.9
	33/28	61.3	141.6	83.7	95.5	
	Mean (±14.7)	24.3	106.2	103.6	78.0	
			(±25.3)		(±14.6)	
	15/10	65.9	104.5	122.2	97.5	
	21/16	59.0	186.6	179.4	141.7	
1500	27/22	40.6	129.3	230.4	133.4	73.9
	33/28	161.9	278.1	250.8	230.3	
	Mean (±12.7)	81.9	174.6	195.7	150.7	
			(±24.1)		(±13.9)	
	15/10	87.9	125.8	150.4	121.4	
	21/16	91.4	205.2	206.3	167.6	
2500	27/22	59.3	185.2	272.2	172.2	70.4
	33/28	225.7	322.5	314.4	287.5	
	Mean (±12.1)	116.1	209.7	235.8	187.2	
			(±22.9)		(±13.2)	
	15/10	105.4	146.1	177.9	143.1	
	21/16	102.1	223.9	233.5	186.5	
3500	27/22	67.6	219.1	303.6	196.8	66.9
	33/28	258.2	337.7	336.1	310.7	
	Mean (±11.5)	133.3	231.7	262.8	209.3	

* Transpiration readings were taken at the day temperature, viz., in the 15/10°C regime at 15°C; in the 21/16°C regime at 21°C; etc.

APPENDIX 5 (cont.)

TABLE A34 P/T^* ratio ($\times 10^4$) at 3500 ft.c.

Temperature regime (°C)	Per cent Shading			Mean (±4)
	0	46 (±7)	74	
15/10	195	171	154	173
21/16	134	104	97	112
27/22	112	93	75	93
33/28	62	53	47	54
Mean (±4)	126	105	93	108
	L.S.D.5% 21	L.S.D.1% 29	L.S.D. .1% 38	

* Where P = rate of gross photosynthesis (mg CO_2/c.c./hr) = rate of net photosynthesis + dark respiration rate

T = transpiration rate (mg H_2O/c.c./hr)

Note: P and T were measured at the day temperature, viz., in the 15/10°C regime at 15°C; in the 21/16°C regime at 21°C; etc.

D. Dry matter production : 8-month-old P. radiata seedlings

TABLE A35 Shoot O.D.Wt. (g)

Temperature regime (°C)	Per cent Shading			Mean (±1.37)
	0	46 (±2.38)	74	
15/10	20.68	13.68	8.40	14.25
21/16	36.66	26.40	12.79	25.28
27/22	33.18	16.39	6.10	18.55
33/28	16.15	12.50	3.66	10.77
Mean (±1.19)	26.67	17.24	7.74	17.22
	L.S.D.5% 6.84	L.S.D.1% 9.17	L.S.D. .1% 12.10	

APPENDIX 5 (cont.)

TABLE A36 Root O.D.Wt. (g)

Temperature regime (°C)	Per cent Shading			Mean (±0.39)
	0	46 (±0.68)	74	
15/10	8.00	3.70	1.94	4.54
21/16	15.82	7.19	2.78	8.59
27/22	12.62	3.75	1.39	5.92
33/28	5.04	2.22	0.55	2.60
Mean (±0.34)	10.36	4.21	1.66	5.41
	L.S.D.5% 2.09	L.S.D.1% 2.94	L.S.D. .1% 4.15	

TABLE A37 Whole plant O.D.Wt. (g)

Temperature regime (°C)	Per cent Shading			Mean (±1.99)
	0	46 (±0.68)	74	
15/10	28.67	17.38	10.34	18.79
21/16	52.48	33.59	15.56	33.87
27/22	45.80	20.14	7.49	24.47
33/28	21.19	14.72	4.21	13.37
Mean (±1.72)	37.03	21.46	9.40	22.63
	L.S.D.5% 10.61	L.S.D.1% 14.88	L.S.D. .1% 21.03	

APPENDIX 5 (cont.)

TABLE A38 Root/shoot ratio

Temperature regime (°C)	Per cent Shading			Mean (±0.01)
	0	46 (±0.01)	74	
15/10	0.39	0.27	0.23	0.30
21/16	0.43	0.27	0.22	0.31
27/22	0.38	0.23	0.23	0.28
33/28	0.31	0.18	0.16	0.22
Mean (±0.01)	0.38	0.24	0.21	0.28
	L.S.D.5% 0.04	L.S.D.1% 0.06	L.S.D. .1% 0.08	

APPENDIX 6

COMPARISON OF THE MORPHOLOGICAL CHARACTERISTICS OF 'SUN' AND 'SHADE' PLANTS

Morphological feature	Comparison - sun vs. shade	Some references
1. Basal stem diameter	Thicker	Shirley, 1945 Daubenmire, 1947
2. Internodes	Shorter	Daubenmire, 1947
3. Branching	More prolific	Daubenmire, 1947 Cameron, 1964
4. Angle of orientation of leaf to incident light	Smaller	Daubenmire, 1947 Grime, 1965
5. Ratio of total leaf area to vascular tissue of supporting stem	Lower	Daubenmire, 1947
6. Stomata	Smaller and more densely packed. Outer stomatal chamber in Pinaceae deeper and more rows of stomata/leaf	Helmers, 1943 Daubenmire, 1947
7. Leaf cells	Smaller, walls thicker, particularly near upper surface	Gourley and Nightingale, 1921 Daubenmire, 1947
8. Leaf size	Smaller due to 7	Shirley, 1936 Grime, 1965
9. Leaf thickness	Usually thicker due to 15, 21, and 22	Chitashvili, 1963 Evenari et al., 1965
10. Walls of chlorenchyma cells (Pinaceae)	Less folded. These folds and ridges which are characteristic of pine, project into the interior of cells thus allowing more chlorophyll granules to align themselves along the cell walls	Coulter and Chamberlain, 1910 Daubenmire, 1947
11. Chloroplasts	Smaller and fewer	Daubenmire, 1947 Fogg, 1963
12. Cuticle	Thicker, with heavier wax secretions	Eames and McDaniels, 1947 Eglinton and Hamilton, 1967
13. Epidermis	Thicker. Lateral cell walls less wavy in outline	Helmers, 1943 Cameron, 1964
14. Hypodermis	Thicker usually. Composed of water layer and sclerenchymatous cells which give rigidity to the pine leaf	Coulter and Chamberlain, 1910
15. Palisade tissue	Better developed and more compactly arranged. Sometimes extra layer of cells	Chitashvili, 1963 Björkman et al., 1965
16. Sponge mesophyll	More compact in sun. Much less reduced by shading than is palisade tissue. The division from palisade to sponge mesophyll is much more distinct in sun leaves	Gourley and Nightingale, 1921 Kramer and Kozlowski, 1960
17. Intercellular spaces	Smaller and fewer in cross-section	Daubenmire, 1947 Björkman et al., 1965
18. Ratio of internal cell surface to external leaf surface	Larger, due to 8 and 9	Turrell, 1936, 1944 Daubenmire, 1947
19. Resin canals (Pinaceae)	More/needle. Generally two in P. radiata - medially arranged	Sutherland, 1933 Helmers, 1943
20. Endodermis	Well lignified in sun. Nil to slight lignification in shade	Larsen, 1927
21. Vascular system	Better developed. Large stele in proportion to non-vascular tissue. Bundles usually closer together in sun foliage	Helmers, 1943 Kramer and Kozlowski, 1960
22. Xylem tissue	Greater proportion of xylem on cross-sectional basis, i.e., more water conducting tissue	Shirley, 1936 Helmers, 1943
23. Root development	Longer, more numerous, and more branched	Daubenmire, 1947
24. Shoot dry matter production	Higher	Daubenmire, 1947 Evenari et al., 1965
25. Root dry matter production	Higher	Daubenmire, 1947 Burschel and Huss, 1964
26. Root/shoot ratio	Higher	Daubenmire, 1947 Bormann, 1958

APPENDIX 7

ESTIMATION OF SOIL MOISTURE CONTENT (% of O.D.Wt.) IN UNDRAINED POTS CONTAINING SEEDLINGS

Legend:
- SWC = weight of water in soil - g
- SMC = soil moisture content - % of O.D.Wt.
- A = weight of pot = 426g
- B = weight of O.D. soil = 3184g
- C = total fresh weight of plant = (D+J)
- D = fresh weight of shoot - g
- E = moisture content of shoot - %
- F = O.D. weight of root - g
- G = relative moisture content of root/shoot
- H = moisture content of root = (ExG) %
- J = fresh weight of root = $F\ \frac{(H+100)}{100}$ g
- T = total weight of potted plant - g

Now
$$SWC = T - [A+B+C]$$
$$= T - [426+3184+ (D+J)]$$
$$= T - [3610 + D + (\frac{F(H+100)}{100})]$$
$$= T - [3610 + D + \frac{F((ExG)+100)}{100}]$$

and as T, D, F, E, and G are known, SWC can be calculated.

Then,
$$SMC\% = \frac{SWC}{3184} \times 100$$

APPENDIX 8

SPATIAL DISTRIBUTION OF CHLOROPHYLL IN THE CROWN OF A YOUNG P. RADIATA TREE

TABLE A39 Spatial variation in the concentration of chlorophyll on a surface area (mg/dm^2) and volume (mg/cm^3) basis and in the Ca/Cb ratio in the crown of a 6 1/3-year-old plantation grown P. radiata tree. Sampled 9/11/65 during a severe drought. Total tree height 18'.

Sample no.	Orientation	Needle age (yrs)	Height (ft)	Chlorophyll-a		Chlorophyll-b		Chlorophyll-a+b		Ca/Cb
				mg/dm^2	mg/cm^3	mg/dm^2	mg/cm^3	mg/dm^2	mg/cm^3	
5	NE	1	4.0	1.33	1.04	0.57	0.44	1.90	1.48	2.33
8	S	1	4.0	1.24	1.17	0.55	0.52	1.80	1.69	2.23
14	SW	1	6.0	1.68	0.97	0.66	0.38	2.35	1.35	2.54
11	NE	1	8.0	1.78	1.02	0.75	0.43	2.53	1.45	2.37
19	SSW	1	9.0	2.02	1.05	0.79	0.41	2.81	1.46	2.56
17	NE	1	10.5	2.10	1.01	0.81	0.39	2.91	1.40	2.60
22	SSE	1	13.5	2.23	0.98	0.93	0.41	3.17	1.38	2.39
21	NNE	1	14.0	2.09	0.88	0.91	0.38	3.00	1.26	2.30
2	NE	2	2.0	1.07	0.84	0.50	0.40	1.58	1.24	2.13
4	SSE	2	2.0	0.96	0.75	0.50	0.39	1.47	1.14	1.92
6	NE	2	3.3	1.24	0.77	0.52	0.33	1.77	1.10	2.34
9	S	2	3.3	1.28	0.75	0.60	0.35	1.89	1.10	2.12
15	SW	2	5.0	1.66	0.90	0.68	0.37	2.34	1.28	2.44
12	NE	2	6.3	1.64	0.84	0.71	0.37	2.35	1.21	2.28
20	SSW	2	7.3	1.90	0.84	0.83	0.37	2.74	1.20	2.29
18	NE	2	8.5	1.83	0.84	0.71	0.33	2.54	1.16	2.57
7	NE	3	2.8	1.25	0.65	0.58	0.31	1.84	0.96	2.13
16	SW	3	4.5	1.37	0.74	0.59	0.32	1.96	1.06	2.31
13	NE	3	5.5	1.39	0.70	0.62	0.32	2.01	1.02	2.23
3	NE	4	1.5	1.06	0.59	0.53	0.30	1.59	0.89	2.00
10	S	4	2.8	1.21	0.65	0.61	0.32	1.82	0.97	1.99
23	*	1	15.0	2.45	0.94	0.99	0.40	3.44	1.32	2.48
24	*	2	9.5	2.08	0.73	0.87	0.31	2.96	1.03	2.39
25	*	3	6.0	1.39	0.62	0.61	0.27	2.00	0.89	2.26

* Stem fascicles

APPENDIX 8 (cont.)

TABLE A40 Spatial variation in the concentration of chlorophyll on a fresh weight (F.Wt.) and oven dry weight (O.D.Wt.) basis (mg/g) in the crown of a 6 1/3-year-old plantation grown P.radiata tree. Sampled 9/11/65 during a severe drought. Total tree height 18'.

Sample no.	Orientation	Needle age (yrs)	Height (ft)	Chlorophyll-a		Chlorophyll-b		Chlorophyll-a+b	
				F.Wt.	O.D.Wt.	F.Wt.	O.D.Wt.	F.Wt.	O.D.Wt.
5	NE	1	4.0	0.83	2.30	0.35	0.98	1.18	3.28
8	S	1	4.0	0.90	2.42	0.40	1.08	1.31	3.50
14	SW	1	6.0	0.93	2.39	0.37	0.94	1.30	3.34
11	NE	1	8.0	0.91	2.60	0.38	1.10	1.30	3.70
19	SSW	1	9.0	0.98	2.49	0.38	0.97	1.37	3.46
17	NE	1	10.5	0.93	2.42	0.36	0.93	1.29	3.35
22	SSE	1	13.5	0.98	2.57	0.41	1.08	1.38	3.65
21	NNE	1	14.0	0.98	2.52	0.43	1.10	1.41	3.62
2	NE	2	2.0	0.77	2.04	0.36	0.96	1.13	3.00
4	SSE	2	2.0	0.69	2.06	0.36	1.07	1.05	3.13
6	NE	2	3.3	0.81	2.09	0.34	0.89	1.15	2.98
9	S	2	3.3	0.74	2.09	0.35	0.98	1.10	3.08
15	SW	2	5.0	0.85	2.12	0.35	0.87	1.20	2.99
12	NE	2	6.3	0.83	2.06	0.36	0.90	1.19	2.97
20	SSW	2	7.3	0.86	2.12	0.37	0.93	1.23	3.06
18	NE	2	8.5	0.82	2.00	0.32	0.78	1.14	2.78
7	NE	3	2.8	0.68	1.77	0.32	0.83	1.00	2.60
16	SW	3	4.5	0.73	1.75	0.32	0.76	1.04	2.50
13	NE	3	5.5	0.72	1.76	0.32	0.79	1.05	2.54
3	NE	4	1.5	0.64	1.68	0.32	0.84	0.96	2.52
10	S	4	2.8	0.67	1.86	0.34	0.94	1.00	2.80
23	*	1	15.0	0.93	2.32	0.38	0.94	1.31	3.26
24	*	2	9.5	0.82	2.00	0.34	0.84	1.16	2.84
25	*	3	6.0	0.71	1.65	0.32	0.73	1.03	2.38

* Stem fascicles

APPENDIX 8 (cont.)

TABLE A41 Spatial variation in the concentration of chlorophyll on a surface area (mg/dm^2) and volume (mg/cm^3) basis, and in the Ca/Cb ratio, in the crown of a 7 1/2-year-old plantation grown P.radiata tree. Sampled 20/1/67 following good seasonal conditions. Total tree height 29'.

Sample no.	Orientation	Needle age (yrs)	Height (ft)	Chlorophyll-a		Chlorophyll-b		Chlorophyll-a+b		Ca/Cb
				mg/dm^2	mg/cm^3	mg/dm^2	mg/cm^3	mg/dm^2	mg/cm^3	
11B	N	1	8.3	1.48	0.90	0.83	0.50	2.31	1.40	1.79
17B	NE	1	11.5	1.89	0.95	1.00	0.50	2.89	1.45	1.90
21A	NNE	1	15.0	2.32	0.98	1.37	0.58	3.69	1.56	1.69
26	N	1	24.0	2.05	0.79	1.21	0.46	3.26	1.25	1.70
5 ø	N	2	3.8	1.13	0.90	0.65	0.52	1.78	1.41	1.73
11 ø	N	2	8.0	1.67	0.95	0.96	0.54	2.63	1.50	1.74
17 ø	NE	2	10.5	1.74	0.80	0.92	0.42	2.66	1.22	1.90
21 ø	NNE	2	14.0	2.04	0.90	1.14	0.50	3.18	1.40	1.78
2 ø	N	3	2.0	1.09	0.93	0.67	0.57	1.76	1.50	1.63
6 ø	N	3	3.3	1.19	0.78	0.66	0.44	1.85	1.22	1.79
12 ø	N	3	6.3	1.47	0.74	0.93	0.47	2.40	1.80	1.58
18 ø	NE	3	8.5	1.44	0.64	0.87	0.38	2.31	1.02	1.66
7 ø	N	4	3.0	1.05	0.54	0.70	0.36	1.75	0.90	1.51
13 ø	N	4	5.5	1.16	0.59	0.75	0.38	1.90	0.97	1.55
3 ø	N	5	1.5	1.05	0.55	0.67	0.35	1.73	0.90	1.57
23A	*	1	27.0	2.34	0.75	1.17	0.38	3.51	1.13	2.00
23B	*	1	19.5	2.54	0.77	1.32	0.40	3.85	1.18	1.93
23 ø	*	2	15.0	2.09	0.78	1.11	0.41	3.20	1.19	1.89
24 ø	*	3	9.5	1.62	0.58	0.91	0.32	2.54	0.90	1.78
25 ø	*	4	6.0	1.20	0.54	0.81	0.96	2.01	0.90	1.48

ø This foliage was also sampled on 9/11/65 (see Table A39)

* Stem fascicles

APPENDIX 8 (cont.)

TABLE A42 Spatial variation in the concentration of chlorophyll on a fresh weight (F.Wt.) and oven dry weight (O.D.Wt.) basis (mg/g) in the crown of a 7 1/2-year-old plantation grown P.radiata tree. Sampled 20/1/67 following good seasonal conditions. Total tree height 29'.

Sample no.	Orientation	Needle age (yrs)	Height (ft)	Chlorophyll-a		Chlorophyll-b		Chlorophyll-a+b	
				F.Wt.	O.D.Wt.	F.Wt.	O.D.Wt.	F.Wt.	O.D.Wt.
11B	N	1	8.3	0.84	2.28	0.47	1.28	1.31	3.56
17B	NE	1	11.5	0.84	2.29	0.44	1.21	1.29	3.50
21A	NNE	1	15.0	0.92	2.49	0.55	1.47	1.47	3.97
26	N	1	24.0	0.84	2.62	0.49	1.54	1.33	4.16
5 ø	N	2	3.8	0.71	1.91	0.41	1.10	1.11	3.02
11 ø	N	2	8.0	0.85	2.29	0.49	1.31	1.34	3.60
17 ø	NE	2	10.5	0.84	2.13	0.44	1.13	1.28	3.26
21 ø	NNE	2	14.0	0.88	2.25	0.49	1.26	1.37	3.51
2 ø	N	3	2.0	0.71	1.81	0.43	1.11	1.14	2.92
6 ø	N	3	3.3	0.73	1.82	0.41	1.01	1.14	2.83
12 ø	N	3	6.3	0.74	1.77	0.47	1.12	1.21	2.89
18 ø	NE	3	8.5	0.63	1.59	0.38	0.95	1.01	2.54
7 ø	N	4	3.0	0.59	1.44	0.39	0.96	0.98	2.40
13 ø	N	4	5.5	0.62	1.45	0.40	0.93	1.02	2.38
3 ø	N	5	1.5	0.58	1.43	0.37	0.91	0.95	2.34
23A	*	<1	27.0	0.80	2.40	0.40	1.20	1.19	3.61
23B	*	1	19.5	0.82	2.19	0.43	1.13	1.25	3.32
23 ø	*	2	15.0	0.80	1.93	0.42	1.02	1.22	2.95
24 ø	*	3	9.5	0.67	1.63	0.38	0.92	1.04	2.55
25 ø	*	4	6.0	0.54	1.20	0.36	0.81	0.90	2.02

ø This foliage was also sampled on 9/11/65 (see Table A40)

* Stem fascicles

APPENDIX 9

TABLE A43 Spatial variation in the relative water content (RWC) of foliage sampled from the crown of a 7-year-old P. radiata tree 24' tall

Spring whorl (year)	Sample no.	Ht. of whorl (ft)	Sample height (ft)	Needle age (yrs)	Relative water content - %	
					Field *	Laboratory +
1961	1	1.5	2.0	1	88.8	92.6
	2		2.0	2	86.6	89.0
	3		1.5	3	85.3	85.9
	4		1.5	4	83.8	83.0
1962	5	4.5	7.0	1	92.2	94.6
	6		6.5	2	88.1	90.0
	7		6.0	3	85.0	85.6
	8		5.5	4	80.5	82.1
1963	9	7.0	11.5	1	92.2	93.8
	10		10.5	2	88.9	89.7
	11		9.0	3	86.0	84.6
	12		7.5	4	81.2	83.7
1964	13	10.0	15.5	1	92.7	94.0
	14		14.0	2	88.2	91.1
	15		12.0	3	85.9	89.4
1965	16	14.5	20.0	1	88.5	95.4
	17		17.5	2	87.1	93.3
1966	18	18.0	19.0	1	91.1	91.8

* The field measurement of RWC was undertaken on 17/8/67 between 0710-0736 hrs A.E.S.T.

\+ The laboratory measurement of RWC was made at the time of measurement of gas exchange of detached shoots (see Appendix 9, Table A44).

APPENDIX 9 (cont.)

TABLE A44 Spatial variation in the photosynthetic capacity of foliage detached from the crown of a 7-year-old P. radiata tree 24' tall. Temperature - 25 ± 1°C.

Legend: (1) Spring whorl - year (2) Sample no. (3) Height of whorl - ft (4) Height of sample - ft (5) Needle age - years (6) Dark respiration - mg CO_2/c.c./hr (7), (8), (9), (10) Net photosynthesis at 500, 1500, 2500, and 3500 ft.c. respectively - mg CO_2/c.c./hr (11) Compensation point for light - ft.c. (12) Net photosynthesis at light saturation - mg CO_2/c.c./hr (extrapolated) (13) Relative water content of foliage - %.

1	2	3	4	5	6	7	8	9	10	11	12	13*
1961	1	1.5	2.0	1	0.09	0.22	0.26	0.22	0.20	105	0.27	92.6
1961	2	1.5	2.0	2	0.09	0.17	0.25	0.25	0.25	130	0.25	89.0
1961	3	1.5	1.5	3	0.09	0.33	0.47	0.44	0.42	85	0.47	85.9
1961	4	1.5	1.5	4	0.17	0.01	0.03	0.04	0.05	465	0.05	83.0
1962	5	4.5	7.0	1	0.16	0.28	0.35	0.35	0.35	145	0.35	94.6
1962	6	4.5	6.5	2	0.18	0.22	0.71	0.84	0.82	220	0.84	90.0
1962	7	4.5	6.0	3	0.17	0.08	0.16	0.17	0.20	280	0.20	85.6
1962	8	4.5	5.5	4	0.17	-0.08	-0.05	-0.03	-0.01	3800	0.01	82.1
1963	9	7.0	11.5	1	0.22	0.25	1.12	1.41	1.47	135	1.47	93.8
1963	10	7.0	10.5	2	0.25	0.30	1.20	1.57	1.69	125	1.70	89.7
1963	11	7.0	9.0	3	0.22	0.12	0.58	0.68	0.69	315	0.69	84.6
1963	12	7.0	7.5	4	0.33	0.03	0.18	0.20	0.20	560	0.20	83.7
1964	13	10.0	15.5	1	0.27	0.09	0.77	1.13	1.24	375	1.26	94.0
1964	14	10.0	14.0	2	0.40	0.02	0.77	1.18	1.37	480	1.44	91.1
1964	15	10.0	12.0	3	0.33	0.01	0.56	0.82	0.92	490	0.95	89.4
1965	16	14.5	20.0	1	0.33	0.00	0.77	1.13	1.33	410	1.40	95.4
1965	17	14.5	17.5	2	0.40	-0.04	0.64	0.95	1.09	555	1.13	93.3
1966	18	18.0	19.0	1	0.34	0.08	0.84	1.24	1.50	410	1.70	91.8

* RWC measured on foliage at the time of measurement of CO_2-exchange.

APPENDIX 10

SHORT WAVE RADIATION WITHIN THE CROWN OF A *P. RADIATA* PLANTATION SAPLING

The site of the study was a 7-year-old *P. radiata* plantation in Uriarra State Forest, A.C.T. Spacing was nominally 8'x8'. The stand was fully stocked and the canopy was closing. Observations were made on an overcast day (4/10/1966) between 1030-1530 hours A.E.S.T. Short wave radiation was detected with two Kipp and Zonen solarimeters, one fixed in position at the top of the canopy (27' above ground) and the other mobile within the canopy (Figure A2, Plate 9). Radiation detected within the crown of the tree studied is expressed as a percentage of that detected at the top of the canopy (Figure A3).

FIGURE A2 Diagrammatic representation (not to scale) of the method used to record short wave radiation inside the crown of a P. radiata plantation tree relative to that at the top of the canopy.

Legend:

A - 'Olympic' S5178 1/.028 single bell wire PVC insulated cable

B - Kipp and Zonen XR4 recording millivoltmeter

C - Two-way switch

D - Fixed position Kipp solarimeter

E - Cleat jamb

F - 60' nylon cord

G1 and G2 - FG-12 'Fico' pulleys. G1 fixed. G2 detachable

H - Hooks for supporting G2

J - Swinging boom

K - Sister clips

L - Mobile Kipp solarimeter

M - Fine steel wire

N - 30' nylon cord graduated at 3' intervals from the level of the Kipp reflecting shield

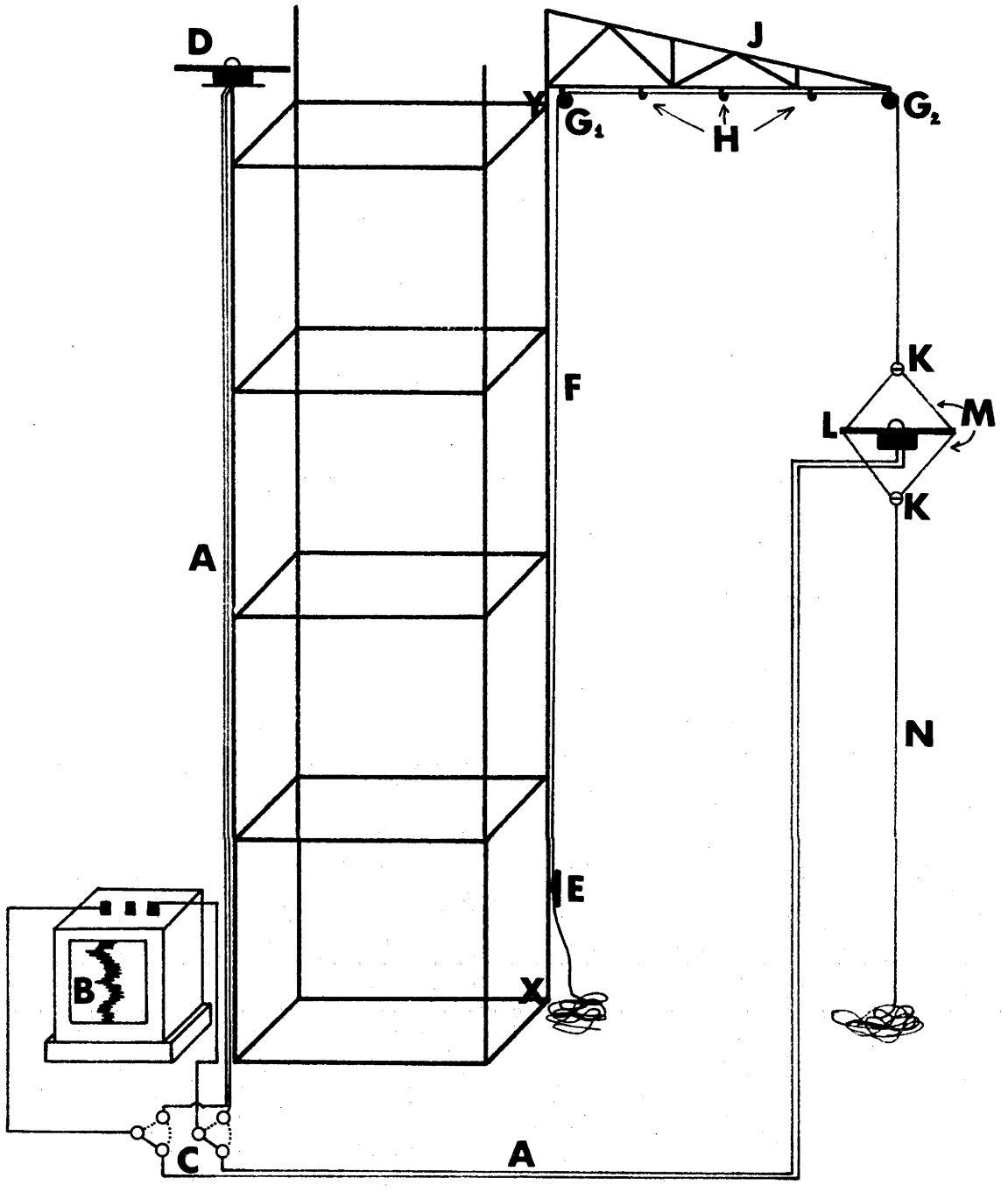

FIGURE A2

PLATE 9 Photograph illustrating the mounting of the 'fixed' Kipp solarimeter on the top of the tower and the suspension of the 'mobile' Kipp from the swinging boom

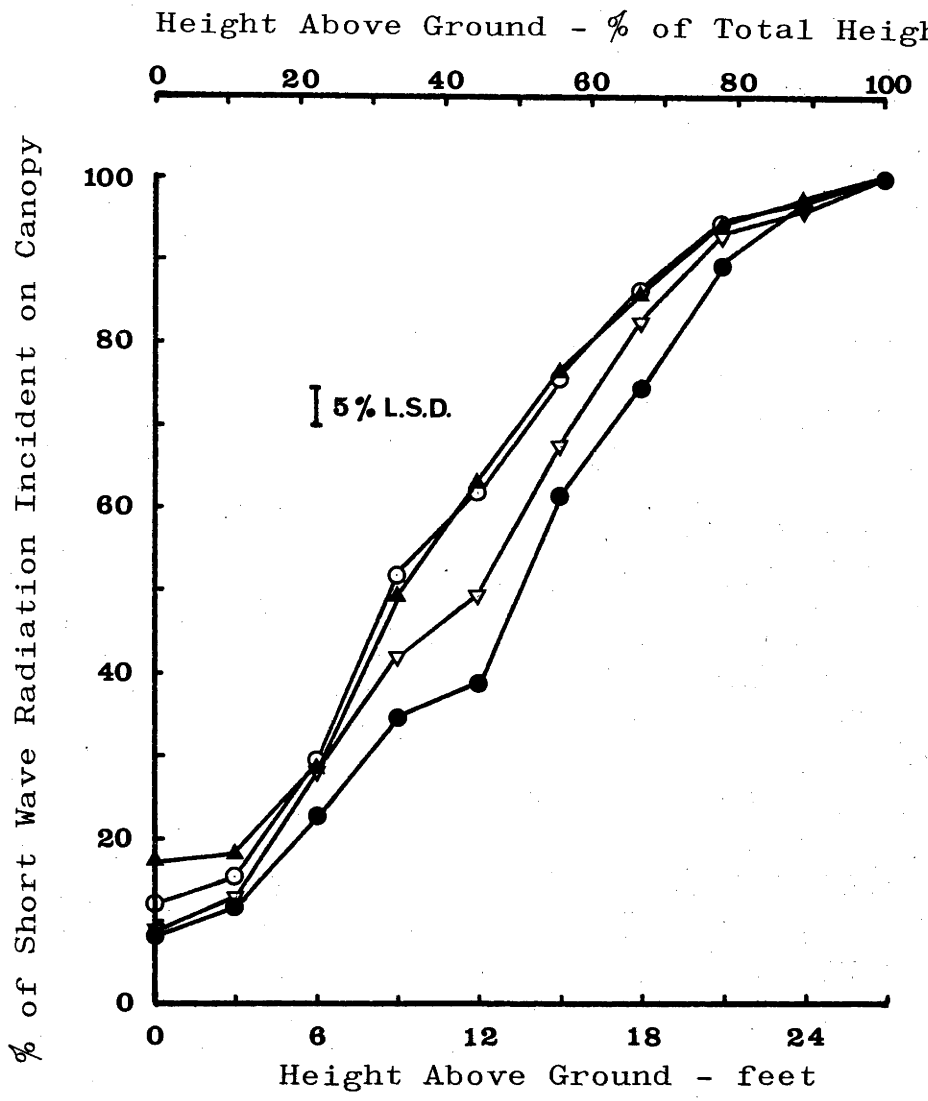

FIGURE A3: Short wave radiation within the crown of a 7-year-old P. radiata plantation tree expressed as a percentage of the radiation detected at the top of the canopy. Observations made on an overcast day (4/10/1966) between 1030 - 1530 hours A.E.S.T. Each point on the graph is an average of six observations.

●——● Sensor 2' from tree bole

▽——▽ " 3' " " "

○——○ " 4' " " "

▲——▲ " 5½' " " "